IMPACT AND MANAGEMENT OF INVASIVE PLANT SPECIES:

A FOOD WEB APPROACH

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A thesis submitted to the University of Bristol in accordance with the requirements of the degree of Doctor of Philosophy in the Faculty of Science

School of Biological Sciences

December 2007

Word Count: 32 381

ABSTRACT

Invasive plants can significantly affect ecosystems. While their impacts on plant populations are widely reported, data on how higher trophic levels are affected by those changes are scarce. The work presented here aims to provide further insights for the study of impacts and management of invasive species, using food webs as a tool for assessing and predicting consequences of habitat disruptions.

The first question I address in this thesis is how the removal of alien plant species from a UK site of conservation interest affects the pollination of a rare plant *Trinia glauca* (Apiaceae). A food web approach was used to identify the main pollinators of this rare plant species, and to understand how this rare plant interacts indirectly with the introduced species present in the study area *via* shared flower visitors. I simulated the likely consequences of removal of alien plants on *T. glauca* pollination, any effects being mediated by shared pollinators. This approach was used to reveal the potential negative impacts of habitat management (here alien plant removal) on the native plants.

Secondly, I consider how the impact of an invasive weed of UK heathlands, *Gaultheria shallon*, propagates through food webs affecting plants, herbivores and parasitoids. Using a quantitative food web approach, I found that diet breadth influences the propagation of impacts, leading to changes in the food web structure. Specialist consumers which had all their resource species affected by *G. shallon* were the most affected by the invasive plant. I consider the implications of these results for conservation ecology and highlight the importance of extending impact evaluation to higher trophic levels as well as considering trophic diversity within levels, for a full evaluation of the consequences of disturbances.

Finally, I evaluated the indirect non-target impacts of a management measure considered safe and environmentally friendly: the use of highly specific weed biocontrol agents. I used 17 replicate food-webs to demonstrate that natural enemies are shared between an abundant successfully established biocontrol agent, *Mesoclanis polana*, and seed herbivore species from native plants. I found that the agent abundance is associated with local insect biodiversity losses, apparent competition being the most likely explanation.

Thus, my study suggests that food webs are a useful tool for assessing and predicting consequences of plant invasion and their management.

DEDICATION

Se há que dedicar a alguém só pode mesmo ser a vocês:

ao meu Pai, a minha Mãe e a ti Eduardo

ACKNOWLEDGEMENTS

Several people have contributed with their help to this thesis. First of all I want to thank my supervisor, Jane Memmott. I really appreciated the guidance, support and encouragement through all these years. I also thank to Yvonne Buckley, who advised on many aspects of this thesis, particularly on the work developed in University of Queensland, Australia. Thanks to Fundação para a Ciência e Tecnologia (Portugal) that has provided the funding for my studentship.

Discussing my doubts and ideas with people was always very helpful. I thank everybody on 'C28' for making our work environment so nice and pleasant and for all the useful discussions. Michael Pocock and Paul Craze for their great patience discussing the statistical analyses during all the thesis; Mariano Devoto, Michael Pocock, Martha Lopezaraiza, Kate Henson, Sarina Pearce, Darren Evans, Ruben Heleno, Rachel Gibson and Jo Brooks, for comments and discussions on the chapters. Interacting with you all was very rewarding.

The help of several people during field and lab work was essential for getting good results. Concerning the work presented in Chapter 2, I would like to thank L. Houston for help on selection of field sites; M. Leivers (Bristol City Council) for permission to work on Avon Gorge; A. Robertson for help identifying *Sorbus* species in Avon Gorge; R. Boada, K. Henson, A. Collings, N. Boase and D. Craven for help in data collection in the Avon Gorge; E. Barbosa for all the help during field work; G. Orledge and C. Collingwood for identification of ant species; J. Deeming, B. Levey and M. Pavott for insect identification; P. Quinn for help with plant identification; M. Pocock for help on statistics; M. Devoto and four anonymous referees for comments on the previous version of the manuscript.

Concerning the work presented in Chapter 3, I would like to thank to T. Branston for permission to work in the field sites and for help locating study areas; T. Branston and I. Cross for information on insect diversity in the study area; H. Kirk, E. Barbosa and R. Torres for field assistance; R. Gibson for help rearing insects; M. Bailey, J. Deeming, G. Broad and A. Polascek for insect identification and information on

parasitoids host range; G. Broad and M. Shaw for information on previous host records; M. Pocock and P. Craze for help on statistics; Y. Buckley, S. Pearce, M. Devoto, M. Pocock and R. Heleno for comments on the manuscript.

Concerning the work presented in Chapter 4, I thank D. Hofmeyer, A. Fay and J. Thomas for information and help defining field sites; P. Edwards and R. Holtkamp for information; E. Barbosa for lab assistance; K. Harris, J. Deeming, G. Daniels, C. Burwell, A. Polascek and M. Buffington for insect identification; J. Dwyer, J. Thomas and Brisbane Botanical Gardens for plant identification; P. Craze and M. Pocock for help on statistics; A. Willis, D. Evans, J. Denslow, L. Hennemman, H. Rebelo, M. Devoto, M. Pocock, N. Jennings, O. Lewis, P. Syrett, R. Morris and R. van Klinken for comments on a previous version of the manuscript. Also a special thanks to Bernard and Dolor for receiving me and helping so much throughout my time in Australia, and to Ilyas whose company in the lab made the long hours of lab work rearing and sorting insect specimens so much nicer.

The friendship and support of many people throughout these years was very important. Thank you Ruben, Martha y José, Kate and Tim, Rachel, Hugo, Miriam, Roberto, Mariano y Natalia, Sarah, Iszy for all your friendship. Special thanks to Martha and Kate whose friendship during nice and not so nice times helped me so much and was particularly important during the first year of my PhD.

Finally, my family, whose encouragement and guidance helped me through all the stages of this long learning process. A special thanks to my father and mother: e para além de tudo desculpas por toda a minha ausência que nunca vou poder recuperar; and to Eduardo, whose support, affection and patience made a big difference.

Obrigado por tudo!

AUTHORS' DECLARATION

I declare that the work presented in this thesis was carried out in accordance with the Regulations of the University of Bristol. The work is original, except where indicated by special reference in the text and no part of the dissertation has been submitted for any other academic award. Any views expressed in the dissertation are those of the author.

Luísa M. G. Carvalheiro

Bristol 21st December 2007

TABLE OF CONTENTS

Chapter 1

INTRODUCTION

Chapter 1

INTRODUCTION

'Over a sufficiently long time scale, all communities arise from a suite of successful invasions from larger spatial arenas'
Robert D. Holt

Impact and management of invasive plants

Invasion of habitats by exotic plants pose one of the major threats to biodiversity, leading to biotic homogenization (McKinney & Lockwood 2005) and to important economic losses (Schmitz & Simberloff 1997; Pimentel *et al.* 2001). Consequently, the causes and impacts of plant invasions have received considerable attention from researchers, with great efforts being made to develop low risk methods of control. However, there is still a lack of knowledge on how invasive species integrate in native communities and how their impacts propagate through food webs.

In this thesis, I use quantitative food webs as a tool for assessing the impact of non-native invasive species, as well as the consequences of their management strategies. I will limit myself to plant and insect communities, considering mutualistic interactions such as pollination, as well as antagonistic interactions such as herbivory and parasitism. In this chapter, I start by presenting a review of the literature, providing the background necessary to deal with questions raised in subsequent chapters. I consider studies that have evaluated how exotic plant species integrate into native plant and insect communities and the impacts arising as a consequence of such integration, as well as studies that have evaluated the risks and impacts of the management measures used to control them. I then briefly review the literature regarding our current

understanding of food web structure and dynamics, highlighting specifically what is known about the insect communities considered throughout this work: the pollinators, the herbivores and the natural enemies of these insects. I end by presenting the aims and objectives of this thesis and providing an outline of its structure.

Causes and consequences of plant invasion

The majority of plant introductions are directly or indirectly caused by man, with the plants either being deliberately introduced for ornamental, food and fibre purposes or accidentally transported to a new country (Riley & Jefferies 2004). A number of these species succeed in establishing in the new environment and, consequently, non-native plant species constitute an important part of the flora in several regions of the world (Pimentel 2002). The frequency of such introductions is likely to escalate with the growing levels of international trade (Levine & D'Antonio 2003). However, only 0.1% of exotic species become invasive (Crawley *et al.* 1996) with many species remaining at low abundances for years or decades after introduction, until its invasion is triggered by a change in environmental conditions (*e.g.* Burke & Grime 1996; Domenech *et al.* 2005). Invertebrate species that directly interact with plants, such as pollinators and herbivores, may also contribute to the success or failure of a plant invasion. For example, if an exotic plant is free from natural enemies' pressure in the introduced range, it may gain a competitive advantage over native species (the Enemy Release Hypothesis (ERH), reviewed by Keane & Crawley 2002).

Although only a small number of exotic plant species become weeds, they invade a wide variety of habitats, including forest plantations, agricultural and natural ecosystems (van Driesche & Bellows 1996), outcompeting native plants (*e.g.* Weiss & Noble 1984a) and reducing plant biodiversity (*e.g.* Hulme & Bremner 2006). Invasive species may also change soil properties (Gordon 1998) such as pH and nutrient content (Ehrenfeld 2003; Feng *et al.* 2007; Isermann *et al.* 2007), sediment movement (Hertling & Lubke 2000; Ndiaye *et al.* 1993), water resources (Stanford & Ward 1993) or soil microbial community composition (Belnap *et al.* 2005; Yu *et al.* 2005). Changes in vegetation structure can also lead to abiotic changes such as in fire regime (van Wilgen & Richardson 1985; Brooks *et al.* 2004) or in the microclimate below vegetation (Lindsay & French 2006). Such disturbances on native plant community and on abiotic

conditions may affect animal species such as soil invertebrates, pollinators, herbivores and parasitoids. For example, Lindsay and French (2006) showed that impacts of changes in microclimate and litter quality caused by one of the invasive plant species studied in this thesis, *Chrysanthemoides monilifera rotundata* (L.) T. Nord (Bitou), led to an increase in abundance of several groups of soil arthropods. Further impacts of this weed were also detected in bird communities, with canopy-feeding generalists and understorey insectivores increasing in abundance and species richness in invaded sites, while birds relying almost exclusively on plant material were negatively affected by the weed (French & Zubovick 1997). Although such studies provide important information on community level impacts, to really understand how an exotic species can impact on other species it is important to identify trophic links between species. Only rarely, however, has it been analysed how invasive plant species affect the trophic relationships that connect species within a community (see the following two papers for exceptions though: Olesen *et al.* 2002; Lopezaraiza-Mikel *et al.* 2007).

Management measures of invasive plant species

Management measures to reduce or remove invasive plants can be grouped in three categories: chemical, mechanical and biological. Which method(s) is/are chosen can have profound effects on the regeneration of native vegetation (Biggerstaff & Beck 2007). For a long time weed management was based around chemical herbicides (Wyse 1994). However, such practices do not always prove effective (*e.g.* Bottrell & Adkisson 1977) and often have non target effects, killing beneficial insects (van Driesche & Bellows 1996), as well as native plants (*e.g.* Matarczyk *et al.* 2002). Mechanical methods have also been used for a long time, although they are considered time and labour demanding (Stocker 1999; Chicouene 2007). Concerns about weed control, soil erosion and water pollution have incentivised the development of new ecologically based alternative methods of weed management (Wyse 1994), such as biological control.

Classical biological control is viewed as a sustainable, cost-effective, environmentally friendly form of pest control (McFadyen 1998). This method is based on the idea that the lack of natural enemies in the introduced range is the main reason for invasiveness. Manipulation of natural enemies' abundances for purposes of pest

control was used traditionally in Asia several thousand years ago (van Driesche & Bellows 1996). Weed biocontrol is, however, a more recent practice, first being used when the cochineal insect *Dactylopius ceylonicus* Green was introduced in northern India to control the pest cactus *Opuntia vulgaris* Miller (Goeden 1978). The most common agents involved in biocontrol of invasive plants are insect herbivores, although other arthropods, fungi, nematodes and vertebrates have also been used (Julien & Griffiths 1998). However, although there are successful examples of weed biological control involving insect herbivores (McFadyen 1998), the majority of the introduced agents are unsuccessful in reaching complete control (*i.e.* when no other method is required to control the target pest) (Hoffmann 1995; McFadyen 1998). This lack of success may be due to agents being unable to establish in the new environment or to the fact that, although established, agents do not lead to a significant effect on weed abundance (Crawley 1989). Consequently, many biological control strategies involve the release of a range of biocontrol agents, with over 400 species of invertebrates or fungi biocontrol agents being released against 133 weed species worldwide (Julien & Griffiths 1998). For example, in an attempt to control one of most widespread weeds of the world, *Lantana camara*, 41 agents were released over 70 countries or regions (Goolsby *et al.* 2006), of which more than half have established, some leading to limited success (Zalucki *et al.* 2007).

The safety of biocontrol is a contentious subject (Fowler *et al.* 2000; Thomas & Reid 2007). One of the main criteria for a species to be considered a safe biocontrol agent is its high host specificity (Fowler *et al.* 2000). Therefore, direct effects on non-target host (see list of known examples in McFadyen 1998) are highly unlikely to occur (Sheppard *et al.* 2006). However, as noted by Willis and Memmott (2005) biocontrol agents can also affect non-target species indirectly, for instance, when the agent shares natural enemies with native species (apparent competition, reviewed by Holt 1977). To understand how these intentionally introduced species are integrated in the diet of native natural enemies, it is important to evaluate the network of trophic interactions of the target ecosystem.

Food webs as a tool for assessing impacts of invasive species

Plant-insect interaction systems can be extremely complex (Memmott *et al.* 2007a), involving dense webs of interactions (*e.g.* Waser *et al.* 1996; Memmott 1999; Muller & Godfray 1999; Morris *et al.* 2004). The complexity and inter-connectivity of species means that an impact on a given species can be easily propagated through the network, potentially affecting trophically distant species. For example, Knight *et al.* (2005) showed that the presence of fish in ponds reduced abundances of larval dragonflies, whose adults feed on pollinators. Thus, plants near ponds with fish receive more pollinator visits than plants near fish-free ponds. There are several other examples of propagation of impacts through food chains, involving a restricted number of species, either from consumer to resources, *i.e.* top-down cascades (*e.g.* Jonsson *et al.* 2007); or from resource to consumer level, *i.e.* bottom-up cascades (*e.g.* Denno *et al.* 2002; Baez *et al.* 2006). By changing soil and plant community properties invasive plants might lead to bottom-up cascades of impacts affecting insect communities that depend on them; while by changing natural enemy communities (*e.g.* Willis & Memmott 2005), biocontrol agents have the potential to cause top-down trophic cascades, leading to impacts on other resources of those natural enemies. Therefore, to understand the full extent of the impact of invasive species, or any other habitat disturbance, studies at a food web scale are needed. The structure of the food web, as well as the characteristics of the type of interactions that link species in a food web, may influence the propagation of the impacts (*e.g.* Dunne *et al.* 2002; Memmott *et al.* 2004). Next, I will briefly summarize what is known about food web structure, and describe the types of interactions that make up food webs, ending by summarising the literature on how food webs have been used in conservation ecology.

<u>Food web structure</u>

Over the last 25 years there has been a great effort made to develop methods to sample, visualize and analyze complex food web structure (Polis & Winemiller 1996; Memmott 1999; Dunne *et al.* 2002; Bersier, *et al.* 2002; Banasek-Richter *et al.* 2004; Cattin *et al.* 2004). As in any other network (*e.g.* neural networks, metabolic networks, social networks and the World Wide Web) food webs consist of a set of nodes (in this case species) which are linked to each other (in this case through trophic relationships) (Newman 2003). However, food webs have specific characteristics that distinguish

them from other networks, such as directionality and stratification (*i.e.* where certain subsets of species are never directly linked with other subsets). Directionality, for example, occurs if caterpillars eat plants, but the reverse situation is impossible. And an example of stratification would be: parasitoids that do not interact directly with plants although they might influence each other through an intermediate subset of species, the caterpillars. Moreover, although, most of food web studies have been based on simple descriptions of 'who eats who', recently it has been recognized that by quantifying interactions, and hence knowing the importance of each resource species to its consumer diet, relevant information on community structure can be gathered (Memmott *et al.* 1994). To describe quantitatively how species are linked between each other, measures such as linkage density, connectance, generability, eveness, nestedness have been developed (Bersier *et al.* 2002; Bascompte *et al.* 2003; Tylianakis *et al.* 2007).

Types of interactions

Interactions among species within a food web can be classified as direct or indirect. Direct interactions in food webs are related to consumption, linking directly two species. Usually, a negative effect of the consumer over the resource is implied, either increasing death rate (*e.g.* effect of parasitoid on its host) or decreasing resource quality/fitness (*e.g.* effect of herbivores on plants). However, in mutualistic relations, such as pollination, consumption may have a positive effect on resources (*e.g.* by feeding on nectar/pollen a pollinator may increase plants reproductive success). Indirect interactions refer to the effects that pass from one species to another *via* one or more intermediary species (Morris & Lewis 2002). There are two basic indirect interactions that regulate propagation of impacts in food webs: exploitative and apparent competition. Exploitative competition occurs when a resource is shared by two consumer species, while apparent competition occurs when two resource species share a common consumer (*i.e.* natural enemy). In the latter case, if an external factor gives advantage to one of the resources increasing its abundance, the consumer abundance will also increase (bottom-up effect), negatively affecting the other resource (top-down effect). Therefore, both resources compete in an indirect way, *via* shared natural enemies (Holt 1977). Exploitative competition has long been accepted as a regulating factor of population (*e.g.* Weinberg 1985). However, the role of apparent competition as a regulating factor has only recently been revealed, with several studies showing strong impacts due to apparent competition on population dynamics, either due to shared

parasites (Tompkins *et al.* 2000), predators (Muller & Godfray 1997) or parasitoids (Morris *et al.* 2001). For example, Morris *et al.* (2004) found that by removing a highly parasitized herbivore species from the studied system, other herbivores had their levels of parasitism reduced and hence increased in their abundance. The potential for apparent competition in a given community can be assessed based on information provided by quantitative food webs (Muller *et al.* 1999).

Use of food webs in an applied conservation context

Only recently, have food webs started to be used in the study of applied questions such as evaluation of the impacts of changes in land management (Gibson *et al.* 2007), of the consequences of climate changes (Memmott *et al.* 2007b) or of the success of restoration programs (Forup & Memmott 2005; Albrecht *et al.* 2007; Forup *et al.* 2007). However, the use of food webs to evaluate impacts (direct or indirect) of invasive plants at a community level is still not common (Zavaleta *et al.* 2001). In this thesis, I will evaluate impacts of invasive species and/or the consequences of their management strategies on two different types of native communities: plant-pollinator community and plant-herbivores-parasitoid community. Literature on what is known on the impacts of invasive species on food webs involving these insect communities is reviewed below.

Invasive plants and plant-pollinator food webs

Invasive plant species are a recognized threat to pollination (Brown & Mitchell 2001; Olesen *et al.* 2002; Memmott & Waser 2002; Morales & Aizen 2006), competing for pollinator service (Rathcke 1983; Waser 1983; Campbell 1985) and altering patterns of insect visitation to native plant species (*e.g.* Ghazoul 2002), leading to a decrease in native plant seed set (Chitka & Schurkens 2001; Brown *et al.* 2002). As a large number of plant species depends on animals for pollination (Buchmann & Nabhan 1996; Richards 1997), the loss of this ecosystem service can have important effects on plant community dynamics (Burd 1994; Ghazoul 2002; Jordano *et al.* 2003) and hence has important consequences for the conservation of plant species (Robertson *et al.* 1999; Allen-Wardell *et al.* 1998; Pauw 2007). Rare plant species with fragmented distribution are a reason for particular concern, as small populations are more likely to

suffer from pollination limitation (Groom 1998; Ghazoul 2006). Moreover, as several agro-ecosystems depend on this 'free' service for crop production (Losey & Vaughan 2006), pollinator decline may also lead to important economic losses (Chacoff & Aizen 2006). Consequently, the conservation of plant-pollinator interactions has received much attention in recent years (*e.g.* Allen-Wardell *et al.* 1998; Kearns *et al.* 1998; Withgott 1999; Kremen & Ricketts 2000; Cane & Tepedino 2001; Kevan & Phillips 2001), with several studies highlighting declines in pollinator communities (Kearns *et al.* 1998; Biesmeijer *et al.* 2006; Holden 2006).

Although plant-pollinator food webs are the most common type of terrestrial food web studied, to my knowledge only three studies have looked at impacts of invasive plants on pollinators at a community scale. Lopezaraiza-Mikel *et al.* (2007) revealed that alien plants may have a beneficial effect for native plants leading to facilitation of pollination; while Oleson *et al.* (2002) found that, in two oceanic islands where both alien plants and pollinators were present, endemic species were more visited than introduced species and that, contrary to expected, alien pollinators did not preferentially visit alien plants. Moreover, Memmott & Waser (2002) showed that alien plants are typically visited by low diverse groups of highly generalist visitors. As pollination networks are highly nested in structure, with generalist pollinators that depend on abundant resources being the main visitors of rare plants (Bascompte *et al.* 2003), abundant plant species (such as invasive plants) might therefore facilitate the pollination of the rarer plants in their communities (Gibson *et al.* 2006).

Other studies have looked at how habitat disturbance can affect pollination systems. For example, Vazquez & Simberloff (2003) showed that changes in vegetation structure caused by introduction of ungulates modified plant population density. Such changes affected the plant pollinator interactions with consequences on the reproduction of an herb species. As pollinator systems have typically several generalist species (Waser *et al.* 1996; Memmott 1999; Olesen & Jordano 2002), and generalist species are known to have lower susceptibility to habitat changes than specialist species (*e.g.* Zabel & Tscharntke 1998; Rand & Tscharntke 2007) they may also be more resilient to linked extinctions (Waser *et al.* 1996) than other more specialized systems, such as herbivory.

Invasive plants and plant-herbivore-natural enemy food webs

Most exotic invasive plants are an unsuitable resource for native herbivores, possibly due to the presence of plant's defensive chemicals to which native herbivores are not adapted (Carpenter & Cappucino 2005). Therefore, by out competing native plants (*e.g.* Hulme & Bremner 2006), invasions may lead to a reduction in herbivore resources, this acting as a limiting factor for herbivore communities (*e.g.* Hellmann 2002; Spencer & Ksander 2004). Changes in the structure of plant communities that occur as a consequence of habitat disturbances may also affect higher levels of the food web. For example, Gratton and Denno (2005) showed that changes in plant community due to invasion by a perennial grass lead to changes in arthropod communities, with loss of species that depended on native vegetation and an increase of those that were able to use the weed as a resource. As species at higher trophic levels are likely to be at higher extinction risk (Cronin 2004), natural enemies may be more affected by disturbances caused by invasive species than herbivores. Therefore, to fully evaluate impacts of invasive species it is important also to consider the natural enemies present in the system. Furthermore, effects at plant level caused by invasive plants, or any other disturbance factor, can affect natural enemy communities directly through changes in vegetation structure or changes in other abiotic habitat characteristics (soil nutrient content, air humidity, shading).

Several studies have looked at herbivore-natural enemy food webs, and a number of published quantitative host-parasitoid webs exist (*e.g.* Memmott *et al.* 1994; Muller & Godfray 1997; Muller *et al.* 1999; Willis & Memmott 2005). Schonrogge & Crawley (2000) used a quantitative herbivore-parasitoid food web to show that the presence of an abundant exotic resource lead to changes of the diet breath of consumers, some becoming highly dependent on the exotic species. Natural enemies can have an important top-down regulating role on herbivore populations (Haukioja 2005; Morris *et al.* 2004) and hence play an important role in structuring host communities (Dobson & Hudson 1986; Minchella & Scott 1991; Mouritsen & Poulin 2002; Montoya *et al.* 2003). This regulatory role is an important ecosystem service (biological control of insects) for both conservation ecology and agriculture, preventing outbreaks of herbivores which may affect plant productivity (Mattson & Addy 1975; Kitchell *et al.* 1979; Holland *et al.* 1992).

Some studies have constructed quantitative plant-herbivore-parasitoid food webs studies in habitats where both native and exotic plant species are present (*e.g.* Henneman & Memmott 2001; Sheppard *et al.* 2004; Willis & Memmott 2005). By presenting quantitative information on the frequency of interactions, such studies provide insights on the potential of each species to influence the whole community and hence could be used to test hypotheses on the abundance, distribution and host specificity of consumer insects (Novotny *et al.* 2004). However, none of the studies mentioned above used quantitative food webs to evaluate impacts of invasive plants on native systems.

Aims of this study

The aim of this study is to use a food web approach to provide insights on how invasive plants are integrated in native communities and to evaluate the consequences of their management measures. Specifically, I am interested in understanding how impacts are propagated and regulated through trophic networks affecting pollinator, herbivore and natural enemy's communities, to consider how food webs can be used to assess and predict impacts of invasive species.

Thesis structure

The purpose of this thesis is to understand how invasive plants, along with the management measures associated with them, influence native communities of plants and insects. In the first chapter, I have provided an overview of plant invasions, their causes and consequences and management measures, ending up by considering food webs as a means of studying their impacts.

In Chapter two, I use food web data on plant-flower visitor interactions, collected in 2004, to characterise the flower visitors of a rare plant of English limestone grasslands, *Trinia glauca*, and to evaluate how this species is integrated in a local food web of native and exotic species. Exclusion experiments on two less endangered populations of *T. glauca*, carried out the following year, tested the effectiveness of the

most common flower visitors as pollinators. I end this chapter by using the initial food web data as a means of creating a testable hypothesis to consider the likely impact of the habitat management recommended for the site.

In Chapter three, I use a natural gradient of a highly invasive weed, *Gaultheria shallon*, in an endangered UK habitat, lowland heathland, to evaluate how impacts of invasive plants propagate up through plant-herbivore-parasitoid food webs. I consider the role of diet breath on the regulation of these trophic cascades, evaluating the predictability of the impacts at higher trophic levels.

In Chapter four I study a highly specific biocontrol agent, *Mesoclanis polana*, that was recently introduced into Australia to control the weed, *Chrysantomoides monilifera* Bitou, and evaluate the indirect effects of biological control. I start by using a food web approach to identify shared natural enemies that may lead to apparent competition, one of the driving forces of community dynamics, and then test the effect of the abundance of the biocontrol agent on the abundance and diversity of plants-seed herbivores-natural enemies' native communities

Finally, in the last chapter (Chapter five) I consider the main findings in my work, discussing the main applications of these and proposing directions for future work.

Chapter 2

POLLINATOR NETWORKS, ALIEN SPECIES AND THE CONSERVATION OF RARE PLANTS: *TRINIA GLAUCA* AS A CASE STUDY

In: **Carvalheiro, L. G.** , Barbosa, E. R. M. & Memmott, J. (*invited resubmission and resubmitted March08*) Pollinator networks, alien species and the conservation of rare plants: *Trinia glauca* as a case study. *Journal of Applied Ecology*.

Chapter 2

POLLINATOR NETWORKS, ALIEN SPECIES AND THE CONSERVATION OF RARE PLANTS: *TRINIA GLAUCA* AS A CASE STUDY

Summary: Despite the essential role of pollination in the maintenance of many rare plant species, conservation management plans rarely consider the service of pollination. This chapter identifies the main pollinators of a rare English plant species, *Trinia glauca* (Apiaceae), and provides recommendations for its conservation.

A community level approach is used, whereby a visitation network is used to identify the direct and indirect links between *T. glauca* and other members of the plant-visitor community at the field site. A field experiment that excluded the main visitor species from female *T. glauca* showed that ants were the main pollinators of *T. glauca*. The network revealed that over the field season 33% of the ants' visits to flowers were to alien plants, with *Cotoneaster horizontalis* making a particularly high contribution (58%) during the *T. glauca* flowering period. The removal of alien plants is a part of the conservation management of *T. glauca* and I simulated the likely consequences of this form of habitat management on *T. glauca* pollination, any effects being mediated by shared pollinators. Although positive or neutral effects are possible, a negative effect is also possible, whereby the removal of alien plants leads to a crash in ant populations, potentially reducing pollinator visits to *T. glauca* by up to 85.2%.

Conserving the pollinators of rare plants is essential if their conservation is to be sustainable in the long term. My data indicate that *T. glauca* is pollinated by ants and demonstrates that, in one of the sites, ants also feed on alien plants, particularly during the flowering season of this rare plant. Based on these results, I suggest that management measures involving removal of alien plants should consider possible negative impacts on rare plants through changes in pollinator populations. In this case, a staged removal is likely to prove the best conservation approach, allowing the pollinators' response to be assessed before any serious negative effects occur.

Key words: ants, food web, alien plants, network, pollination, rare plant, *Trinia glauca*.

Introduction

Pollination plays a key role in the maintenance of genetic variability of plant species (*e.g.* Ohara *et al.* 1996; Paschke *et al.* 2005), and is therefore important in a plant's ability to adapt to environmental change (Lewontin 1974). Unfortunately habitat fragmentation, one of the main threats to biodiversity worldwide, impacts strongly on pollination (Murcia 1996; Valdivia *et al.* 2006) by reducing the diversity and abundance of pollinators (Jennersten 1988; Aizen & Feinsinger 1994) and plant attractiveness as the patch size of flowering plants is reduced (Sih & Baltus 1987). However, there are some populations of rare plants that can maintain stable densities through long periods of time (Lawton 1995; Mace & Kershaw 1997). An understanding of the pollination system of these rare plant species, especially those with fragmented populations, is likely to provide important pointers for the conservation of rare species in general.

Given that most plants use multiple pollinators and most pollinators visit multiple plants (*e.g.* Waser *et al.* 1996), pollination can be viewed at the level of an entire ecological community as a web, or network, of mutually beneficial (mutualistic) interactions between two trophic levels. Plant-pollinator webs, like other types of networks (*e.g.* food webs, neural networks, social networks and the World Wide Web) are composed of a set of nodes (in this case species) which are linked to form a network. Therefore, an impact on a given node can propagate through the network affecting other species. Food web ecologists have developed methods to quantify the robustness of complex networks to the loss of species (*e.g.* Solé & Montoya 2001; Dunne *et al.* 2002; Memmott *et al.* 2004). This approach involves the simulation of primary extinction of species, by removing them from the network, and quantifying any ensuing secondary extinctions, for example, when primary plant extinctions leads to secondary extinction of their specialist pollinators. The amount of extinction that can be tolerated before the network collapses (*i.e.* when all species are lost) can be remarkably small. For example, in a 154 species network, put together by Memmott *et al.* (2000), the simulation of a 10% species loss was sufficient to elicit a cascade of secondary extinctions which led to network collapse (Dunne *et al.* 2004). One of the main limitations of these types of simulations to date though is not considering the potential of adaptation, whereby consumers adjust their diet to the remaining available resources. Generalist species (*i.e.*

species with large diet breaths) would be the most likely to adapt, this both reducing the impact of species loss (Dunne *et al.* 2002; Memmott *et al.* 2004) and increasing the stability of food webs (Kondoh 2003). Moreover, it is also important to consider the possibility of the existence of what Bascompte (2003) describes as 'parametric thresholds'. These enhance the impact of species loss, as extinction could occur before total exhaustion of resources.

While alien species are usually undesirable in areas of high conservation status, they can be linked to many native species through shared pollinators *(e.g.* Olesen *et al.* 2002) and therefore there may be a negative effect on native species following their removal, at least in the short term. With one exception to date (Lopezaraiza-Mikel *et al.* 2007), ecologists have considered only the impact of removing native species from ecological networks. However, using network analysis to predict the impact of removing alien species at the community level could provide data to guide an eradication programme.

My chosen rare plant, *Trinia glauca* (Apiaceae), commonly known as Honewort, is common in several regions of Europe with its distribution limits in South England, Iberian Peninsula and South-west Asia (*e.g.* Constantinidis *et al.* 2002; Nieto-Feliner *et al.* 2003). In England this perennial monocarpic herb species is extremely rare, with a highly fragmented distribution, and is restricted to dry limestone sites (Lovatt 1982) at a few sites in the Bristol/Somerset region (Preston *et al.* 2002). Being dioecious, it is an obligate out-breeder, it is also a poor disperser (Lovatt 1982), which combined with its rarity puts it, theoretically at least, at a high threat of extinction in England. However, the population of this plant has remained relatively stable over many years, and in recent decades there has even been a reported increase in its abundance (Preston *et al.* 2003). Ants are believed to be important pollinators of *T. glauca* (Proctor & Yeo 1973; Lovatt 1982), although this belief is based on observation of visitation (*e.g.* Lovatt 1982), rather than evidence of pollination. Its high dependency on cross pollination (a result of its dioecy) makes pollinator conservation a key part of any management strategy.

The aim of this work is to identify the pollinators of *T. glauca* in England in order to provide better management recommendations. The objectives are threefold: 1) To use a visitation network to quantify and identify *T. glauca*'s flower visitors and to determine how these integrate into the local flower visitation network; 2) To experimentally

exclude putative key pollinators at two sites to determine their role in the pollination of *T. glauca*; 3) To use a species loss simulation to predict the impact of removing alien plant species on *T. glauca's* reproductive success and to use this information to make management recommendations.

Methods

Field Sites

Three of the seven populations of *Trinia glauca* recorded in England (Preston *et al.* 2002) were studied during this research. Visitation data were gathered in 2004 in the Avon Gorge (Ordnance Survey grid reference ST5674), an iconic field site well known for its rare plant populations (Marren 1999). Although one year of visitation data does not capture interannual variability, it does provide a detailed snapshot of how alien plants are integrated in the native flower visitation network. The field experiment was run in 2005 at two sites: Crook Peak (ST387558), an area of semi-natural grassland and scrubland and Sand Point (ST315658), a small area of a south-facing headland with maritime grassland and scrub. Male plant abundance was higher than female plant abundance with the sex ratio among adult plants being approximately 3:2 at both Crook Peak and Sand Point.

Construction of the visitation network

An area of 1480m^2 was selected in the Avon Gorge field site covering a broad range of flowering plants and enclosing the site's entire population of *T. glauca*. A total of 11 survey visits were carried out from 10th May to 27th September 2004, this period covering the main period of insect activity in England. Flower and insect surveys took place approximately every 14 days under dry conditions. In each survey, a stratified random design was used to select 1m^2 quadrats in the study area. The area was divided into nine sub-areas based on habitat type and accessibility. Each sub area was divided into 1m^2 quadrats and 2.5% of these quadrats were randomly selected per sampling occasion. This resulted in 37 1m^2 quadrats chosen for every sampling survey. In each

quadrat, the number of floral units of each plant species was recorded, a floral unit being defined as a unit that a small bee (c. 1cm length) would have to fly between, rather than walk between (Saville 1993). Once the floral survey was complete, each flowering plant species present in the study area was observed for visitation by insects for 20 minutes. On consecutive sampling occasions, plant species were rotated through three time slots, the morning (09h00-12h00), early afternoon (12h00-15h00) and late afternoon (15h00-18h00), to allow each species to be equally observed over time. At least two floral units were observed per plant species per sample. All flower-visitor interactions were recorded, and all visitors were collected for identification, according to standard pollination network techniques (Memmott 1999; Dicks *et al.* 2002). To estimate the overall abundance of each plant species, the average number of flower units per $1m^2$ quadrat was multiplied by the total area of the study site. To estimate the interaction frequency for each visitor-plant species pair, I divided the total number of visits recorded by the number of flower units observed (per 20minutes) and then multiplied by the total number of floral units in the plot. By collecting the insects I did not allow for repeated visits by the same individual, hence some visitation frequencies may be underestimates. However, collecting specimens is essential for identification of visitor species. Hymenoptera, Diptera, and Coleoptera were identified by taxonomists either to species or to morphospecies, Lepidoptera were identified to species by the authors and Heteroptera and parasitoids were morphotyped by the authors. To estimate the overall abundance of each insect species, I sum the overall number of visits per plant species. The data were used to draw a visitation web using software written in Mathematica™. Finally, given the importance of ants in *T. glauca* pollination (see results), the contribution of alien plants to ants' floral diet in each survey visit was calculated.

The field experiment

Pollinator exclusion

The visitation network (see results) revealed that three ant species made up 85.2 % of the insects visiting *T. glauca* in the Avon Gorge. Vazquez *et al.* (2005) predict that the most common visitors are the likely pollinators, and consider interaction frequency to be an adequate surrogate for the total effect of mutualists in pollination systems.

Consequently, I chose to exclude ants from *T. glauca* in the field experiment. Given that it was impossible to exclude each ant species separately, I excluded all ant species from the experimental plants. In each field site (Crook Peak and Sand Point), 70 female plants were selected for the experiment. Thirty five plants at each site were allocated to an ant exclusion treatment and 35 left as controls. To exclude ants from the experimental plants, a ring of fruit tree grease approximately 15mm wide was painted 5-10 cm away from the base of its stem, prior to flowering. To maintain the effectiveness of the exclusion treatment the ant-excluded plants were checked at least every 10 days, with grease reapplied where necessary. No crawling pollinators other than ants were observed at either of the two sites. The experiment encompassed *T. glauca's* flowering season, which lasted from 12th April to 24th May 2005. At the end of the experiment the number of seeds and the number of unfertilized ovules were counted for each plant to estimate reproductive success and a two-way analysis of variance was applied to logit transformed data to test whether reproductive success varied among treatments and sites.

Insect visitation at Crook Point and Sand Point

Insect visitation to *T. glauca* was observed at both experimental sites to check whether ants were the main visitors here, as well as in the Avon Gorge (see results). Sites were visited once a week, with a total of six visits. A maximum of 25 flowering *T. glauca* female plants were observed for 20 minutes per sampling occasion. To avoid interfering with visiting behaviour, insects contacting the stigmas during the observation periods were only collected after leaving the observed plant, using a net or caught directly into killing tubes lined with paper to prevent transfer of pollen between insects. If a visitor could not be caught, the order of the species was recorded and they were classified as ants (distinguishing between *Lasius* sp. and *Myrmica* sp.) or flying visitors. To quantify the effectiveness of the exclusion treatment, the ant-excluded plants were also observed. All collected insects were identified by taxonomists either to species or to morphospecies. All pollinator observations took place between 9 am and 7 pm. A total of 107 observation periods were carried out on female plants.

Insect pollen loads

To count the number of pollen grains of *T. glauca* transported by each insect, up to 45 specimens per species per field site were collected on female flowers. Although some pollen may have been lost during flower visitation (*i.e.* pollen deposition had occurred), this will have affected all visitors equally. Each collected insect was systematically dabbed with a small cube (approximately 3mm x 3mm x 2mm) of glycerine jelly containing basic fuchin stain (Kearns & Inouye 1993). After sampling the pollen, the glycerine jelly was placed on a microscope slide, melted and covered with a cover slip for analysis. Pollen grains were counted and identified by comparing them to a pollen reference collection made from flowers at the field sites. As a substantial proportion of the data on pollen grains count are zeros the number of *T. glauca* pollen grains, and the number of other species' pollen grains, carried by each visitor species were compared using Poisson regression models corrected for overdispersion, and *F* tests on changes in deviance (Sileshi 2006).

The species loss simulation

While the plant species list from the Avon Gorge is predominantly native, there are a notable number of aliens (see Table S2.1 in Supplementary material). These species all grew in the study plot near to the *T. glauca* population. The removal of these species is currently one of the ongoing management techniques used in the conservation of the Avon Gorge (Higgins 2006). To predict the effect of this management on *T. glauca* pollination, the removal of alien plants was simulated *in silico*, using the subset of the network data that includes all the interactions of visitors to *T. glauca*.

The species loss simulation quantifies how the removal of a given species will affect the other species in the network (Solé & Montoya 2001; Dunne *et al.* 2002). In this network, the removal of alien plants constituted the primary extinctions in this analysis, this being simulated by simply removing all the alien plants from the network data. Given that *T. glauca* visitors have generalized dietary requirements however (see results), it is likely that their visitor species will adjust their diet to fit the available resources following the removal of alien species. Therefore, a mathematical expression that models how each flower visitor species adapts its diet to the remaining resources

was developed. Assuming that: i) there is an equilibrium situation before alien removal, where ants foraging strategy is optimized according to resource conditions (*e.g.* availability of resource, energy supplied by resource), ii) for the remaining resources, these conditions will not change, iii) ants maximum foraging range was detected with the sampling method; the adaptation of ants to the removal of a resource will be mostly dependent on the saturation level of the remaining resources (*i.e.* whether a given plant species can, or cannot support further insect visits). Therefore, the importance of a given remaining unsaturated resource to the consumer's diet, after visitors' foraging strategy has adapted to the removal of alien plants, would be given by the original importance (proportion) of that resource to the consumers' diet plus a fraction of the importance of the lost (removed) alien resources:

$$\begin{cases} A_i = O_i + R \times \dfrac{O_i}{\sum_{i=1}^{n}(O_i)} & \text{, if } i \text{ is unsaturated} \\ A_i = O_i & \text{, if } i \text{ is saturated} \end{cases} \qquad \text{eqn 1}$$

Where A_i is the proportion of a given remaining resource species i in the consumer's diet after the removal of alien plants, O_i is the original proportion of a given remaining resource species i in the consumer's diet, R is the proportion of the removed resource in the consumer's diet, before the removal of alien plants and n is the total number of unsaturated resources. For example, if a given visitor's diet consists of 40% of a native plant species A resources (unsaturated), 10% of a native plant species B resources (unsaturated) and 50% by alien plant resources, after alien plants removal, plant A will make up 80% of the visitor's diet, while plant B will make up 20%. However, if native plant B resources are saturated before alien plant removal, the final outcome will be plant A making up 90% of the visitor's diet, while plant B will remain making up 10%. Although simplistic, this model can be used to simulate a range of scenarios after habitat management that leads to reduction of resource diversity (here removing four species of alien plants from the habitat). Moreover, if all remaining resources are saturated, adaptation will not be possible and consequently the final level of resources will be reduced. If these remaining resources remain below the minimum amount of available resource needed for the persistence of the populations of the species involved

(the parametric threshold, see Bascompte 2003), local consumer's populations may decline. Three scenarios were simulated, following the removal of alien plants:

Scenario 1) None of the native resources available in foraging range is saturated, hence ants forage on the remaining species in the same proportion as they did before, maintaining ant population abundance;

Scenario 2) *T. glauca* is saturated as a flower resource to ants but other native resources are not, hence ants forage on *T. glauca* equally as before but increase the visitation rates to unsaturated species (using those unsaturated resources in the same relative proportion as they used before alien removal), thereby maintaining ant abundance;

Scenario 3) All native resources available in foraging range are saturated, thus removal of alien resources leads to an overall reduction of the resources. If the remaining level of resource is lower than ants' parametric threshold, ant population will decline, leading to local extinction.

While intermediate situations could be simulated depending on which resources are or are not saturated, these three scenarios reveal the range of possible effects on *T. glauca* that the removal of alien plants could elicit.

Results

The visitation network

There is a complex network of interactions between plants and their flower visitors in the Avon Gorge, which links two hundred and sixty species together (81 plant species, 179 insect species and morphospecies). *T. glauca* is linked directly to six species of insects, which link it indirectly to many other species of plants and their insect visitors (Fig 2.1). The species of insect that visited *T. glauca* were: the ants *Lasius alienus* ss Förster (59.3% of visitors), *Formica fusca* L. (18.5%) and *Temnothorax albipennis* Curtis (7.4%), the ant mimicking heteropteran, *Myrmecoris gracilis* R.F. Sahlberg (11.1%), the weevil *Phyllobius roboretanus* Gredler (3.7%) and Thysanoptera (< 0.1%).

Both dominant ant species, *L. alienus* and *F. fusca*, were generalist visitors, visiting 10 and 15 other plant species present at the field site, respectively.

During *T. glauca's* flowering period, an alien, *Cotoneaster horizontalis* Decne (Rosaceae), had a particularly high contribution (57.7%) to the ant's diet (Table 2.1). After *T. glauca's* flowering period, the three dominant floral resources used by ants were a native plant, *Scabiosa columbaria* L. (Dipsacaceae), and two alien plant species, *Centranthus ruber* L. DC (Valerianaceae) and *Cotoneaster franchetti* Bois (Rosaceae).

The field experiment

Pollinator exclusion

The tree grease was 79% effective at excluding ants at Crook Peak and 66% effective at Sand Point, these figures describing the percentage of ant-excluded plants on which ants were never seen. It was impossible to achieve 100% exclusion, as both sites are windy and bridges of vegetation were often blown across the grease barrier. At both field sites, 28 experimental and seven control plants were lost, chiefly by grazing sheep or rabbits. The lower number of lost controls reflected the fact that these were replaceable whereas the experimental plants could not be replaced (as the tree grease needed to be in position for the duration of the field season). Despite the reduced sample size there was a significant decrease in reproductive success between the control and ant-excluded plants (Average reproductive success: Control = 77.3% ± 10.4, Experimental = 54.3% ± 13.9; $F_{1, 101} = 85.9$, $P < 0.0001$, Fig 2.2). There was no effect of site on reproductive success ($F_{1, 101} = 0.1$, $P > 0.05$), and no significant interaction between treatment and site ($F_{1, 101} = 0.8$, $P > 0.05$).

Insect visitation at Crook Peak and Sand Point

At both Crook Peak and Sand Point the ant species *L. alienus* and *Myrmica sabuleti* (Meinert) were the most frequent visitors to female flowers of *T. glauca*. At Crook Peak ants were responsible for 98% of the visits to female flowers and at Sand Point ants were responsible for 80.7% of the visits. Seven other species were observed visiting *T. glauca* female flowers: two dipterans (*Delia pratura* (Meigen) and another unidentified small dipteran which escaped before capture), three coleopterans (*Aphthona euphorbiae*

(Schrank); *Cantharis rustica* Fallen; *Meligethes* sp.) and two hymenopterans (an Andrenidae sp. and a parasitoid wasp). However, with the exception of one of the dipterans, *D. platura*, all other species were recorded on just one or two occasions over the sampling season. Both species of ants as well as *D. platura* also visited male *T. glauca* plants.

Insect pollen loads

There was no significant difference in the number of *Trinia glauca* pollen grains carried by ants and other visitors ($F_{1, 151} = 3.2$, $P > 0.05$, average number of pollen grains (ants) = 45.9 ± 153.9, average number of pollen grains (other visitors) = 121.8 ± 303.6, n (ants) = 129, n (other visitors) = 25). However, ants carried significantly less heterospecific pollen than flying visitors ($F_{1, 151} = 37.3$, $P < 0.0001$, average number of pollen grains (ants) = 1.2 ± 3.2, average number of pollen grains (other visitors) = 21.0 ± 58.2).

The species loss simulation

Alien plants were of considerable importance to the ants' floral diet in the Avon Gorge with thirty three percent of ant visits being to alien plants. The alien species loss simulation was carried out on sub network which described the interactions between *T. glauca*, its visitors and its visitor's other host plants (Fig 2.3a). The outcomes were:

Scenario 1: alien removal affects *T. glauca* positively if all native resources are unsaturated with respect to pollinator visitors. Hence ants use native plants to compensate the loss of alien plants, increasing visitation frequency to all native plants pro rata (Fig 2.3b). This leads to a 106.9% increase in visitation frequency to *T. glauca* flowers.

Scenario 2: alien plants removal has a neutral effect on *T. glauca* if its floral resource is saturated, and hence ants replace alien species by native unsaturated resources other than *T. glauca* (Fig 2.3c). In this case, visitation frequency to *T. glauca* flowers would remain unchanged.

Scenario 3: alien removal affects *T. glauca* negatively as ant populations' crash due to saturation of the remaining resources. Ants cannot compensate the lost resources and this leads to an 85.2% reduction on visitation frequency to *T. glauca* flowers (Fig 2.3d).

Discussion

T. glauca, in common with many plants, is clearly embedded in a complex network of plant-flower visitor interactions at the Avon Gorge field site. The food web approach identified ants as the likely pollinators of this rare plant, this being verified by a field experiment. A species loss simulation approach was used to predict the likely effects of removing alien species (a key management strategy in many nature reserves worldwide) on the rare plant's reproductive success and showed that one potential outcome is an 85.2% reduction in visitation to *T. glauca*. In this section we discuss the implications of the field experiment and of the simulation to the conservation of *T. glauca*, using the results here obtained to suggest management recommendations for this species. I end by considering advantages of using a network approach in the conservation of rare plants.

The visitation network

The visitation network here presented adds to a growing collection of plant-pollinator networks, joining the much smaller subset of data in which species abundance along with the frequency of interactions between the species are quantified. The network shows that six species of insects visited *T. glauca*, with ants making up the majority of these visits. The ant species feed on a broad range of flowering plants and adapted their diet according to availability of the flower resource (Table 2.1). The alien plants comprised a substantial part of ants' diet, especially during the *T. glauca* flowering season. Since these alien species are very nectar rich and given that nectar is one of the most important components of a number of ant species' diet (Ricogray 1993, Koptur &

Truong 1998), it is possible that the removal of aliens will have at least a short term impact on ant populations.

The field experiment

Although ants are often considered ineffective pollinators (*e.g.* Beattie *et al.* 1985, Peakall & Beattie 1991), the reduction in *T. glauca* reproductive success clearly shows that they are important pollinators of this plant. There are other examples of pollination by ants especially in plant species with a low, dense flowering stature (Gomez & Zamora 1992; Gomez *et al.* 1996; Gomez 2000). However, due to the relatively small radius of movement around their nests, pollen transfer by ants can lead to a restricted gene flow (Peterson *et al.* 2002). Consequently, sporadic visits by flying visitors, such as bees and flies, may be very important for the maintenance of genetic diversity within a plant population. *Delia platura* (Diptera: Anthomyiidae) was the only flying visitor species whose frequency of visitation was sufficiently high at the field sites for it to be considered a potential pollinator. Even so, it was still a rare visitor, being recorded in only one of the sites (Sand Point) and with all its visits recorded in one single day. In contrast ants were reliable visitors, being present at all three field sites and visiting *T. glauca* throughout its flowering season. Furthermore, flying visitors' pollen loads contained significantly more heterospecific pollen grains in comparison to ants and the deposition of foreign pollen on stigmas by flying visitors could negatively affect pollination (*e.g.* Brown & Mitchell 2001).

Even allowing for the fact that ant exclusion treatment was not 100%, the reproductive success obtained when ants are excluded was surprisingly high (51.2% at Crook Peak and 56.8% at Sand Point), particularly when considering that the plant is reported to be dioecious. Therefore, some of the seeds must be pollinated by other means. The very low number of flying visitors makes it unlikely that these are responsible for the remainder of the pollination. Given that members of the plant family Apiaceae do not possess the characteristics of Lepidoptera pollinated flowers (Proctor *et al.* 1996), nocturnal visitors such as moths are unlikely to be making up the difference. The two *T. glauca* populations at the experimental sites were located in steep and windy areas and were at a high population density, with male and female plants adjacent and it

is possible that wind or the physical brushing together of flowers under windy conditions is leading to pollination at the two experimental sites. However, the *T. glauca* population in Avon Gorge is located in a sheltered gully and plants are sparsely distributed, therefore it is likely to be much more dependent on its pollinators for reproduction. Another possible explanation for the unexpectedly high reproductive success is the occurrence of agamospermy (the asexual formation of embryos and seeds without the occurrence of fertilization), which according to Plitmann (2002) is a common occurrence in many angiosperm taxa, including Apiaceae, albeit unrecorded for *T. glauca*. Interestingly, although the control plants had higher reproductive success, it never reached 100%, which may be due to pollen limitation or limitation due to other ecological factors, such as soil nutrients.

The species loss simulation

The species loss simulation revealed how *T. glauca* could be affected by the removal of alien plants from Avon Gorge, those effects being mediated indirectly by shared pollinators. Further field experiments are needed to determine which of the three scenarios actually occurs in the field and these could be done within the programme of alien plant removal currently running in the Avon Gorge, to the advantage of both pure and applied ecologists. Ideally such experiments should include measuring the impact of alien plant removal on *T. glauca* seed production and recruitment in the short (immediately after alien removal) and the long term (after several insect generations). This type of approach could prove very useful in an applied ecology context, providing a better assessment of the risks and benefits of a given conservation strategy.

Implications for the management of Trinia glauca

Given that *T. glauca* is a rare plant species with a highly fragmented pattern of distribution in England, it could be at high risk of extinction (Lawton 1995; Aguilar *et al.* 2006; Pocock *et al.* 2006) and the maintenance of its pollinators is essential if its conservation is to be sustainable in the long term. As alien plants represent an important

component of the ants' floral diet, the maintenance of these aliens may be important for *T. glauca* populations in the short term. While I am not suggesting that removing the aliens is an inappropriate management tool, I suggest that their impact on pollinators is at least considered, as without the pollinators, the conservation of the rare plants in the Avon Gorge cannot be considered a sustainable conservation programme. Management plans that involve removal of alien plants need to consider the unintended, indirect, short term negative impact, as well as the intended long term positive gains. In the case of *T. glauca*, a cautious approach would be recommended whereby part of the alien plant population is removed and ant behaviour and abundance are closely monitored. This approach would enable "beneficial" insects, such as the ants, that currently rely on the aliens to ecologically adapt to the change of resources.

Concluding remarks

Pollinators are undergoing a widespread decline in Europe and this is believed to be leading to related declines in insect pollinated plants (Biesmeijer *et al.* 2006). Consideration of the importance of pollinators in conservation management plans is a laudable aim, but one that is rarely implemented in practice (Memmott *et al.* 2007a). Here, I have shown that ants are effective and important pollinators in the reproduction of a rare plant. A network approach is a novel tool in conservation research and management practices, but one that could prove very useful. The network also identified likely pollinators and made predictions concerning the consequences of management practices at the site. Testing the predictions provided by a network approach using rigorous experiments (which would be possible within many conservation programmes involving removing alien species) is likely to provide new perspectives on the management of both natural and managed ecosystems.

Tables and Figures

Table 2.1 The flowering plant species used by the two main visitors of *Trinia glauca* in Avon Gorge (*Formica fusca* and *Lasius alienus*), through the main general flowering season (May to September). The figures in the table are the number of ants calculated to be visiting each flower species over the field season. The symbol '-' represents months when the plant species was not flowering.

	early May	mid May	early Jun	mid Jun	early Jul	mid Jul	early Aug	mid Aug	early Sep	mid Sep	end Sep	Total
Native resources:												
Trinia glauca (L.) Dummort	35	51	-	-	-	-	-	-	-	-	-	**86**
Other native plant species	25	129	270	146	47	27	1	1	2	0	1	**649**
Alien resources:												
Centranthus ruber (L.) DC	-	-	159	-	-	-	-	-	-	-	-	**159**
Cotoneaster franchetti Bois	-	-	10	8	69	31	-	-	-	-	-	**118**
Cotoneaster horizontalis Decne	81	-	-	-	-	-	-	-	-	-	-	**81**
Smyrnium olusatrum L.	1	1	-	-	-	-	-	-	-	-	-	**2**
Total contribution of alien plants to ant species diet (%)	57.7	0.60	38.5	5.20	59.5	53.4	0	0	0	0	0	**32.9**

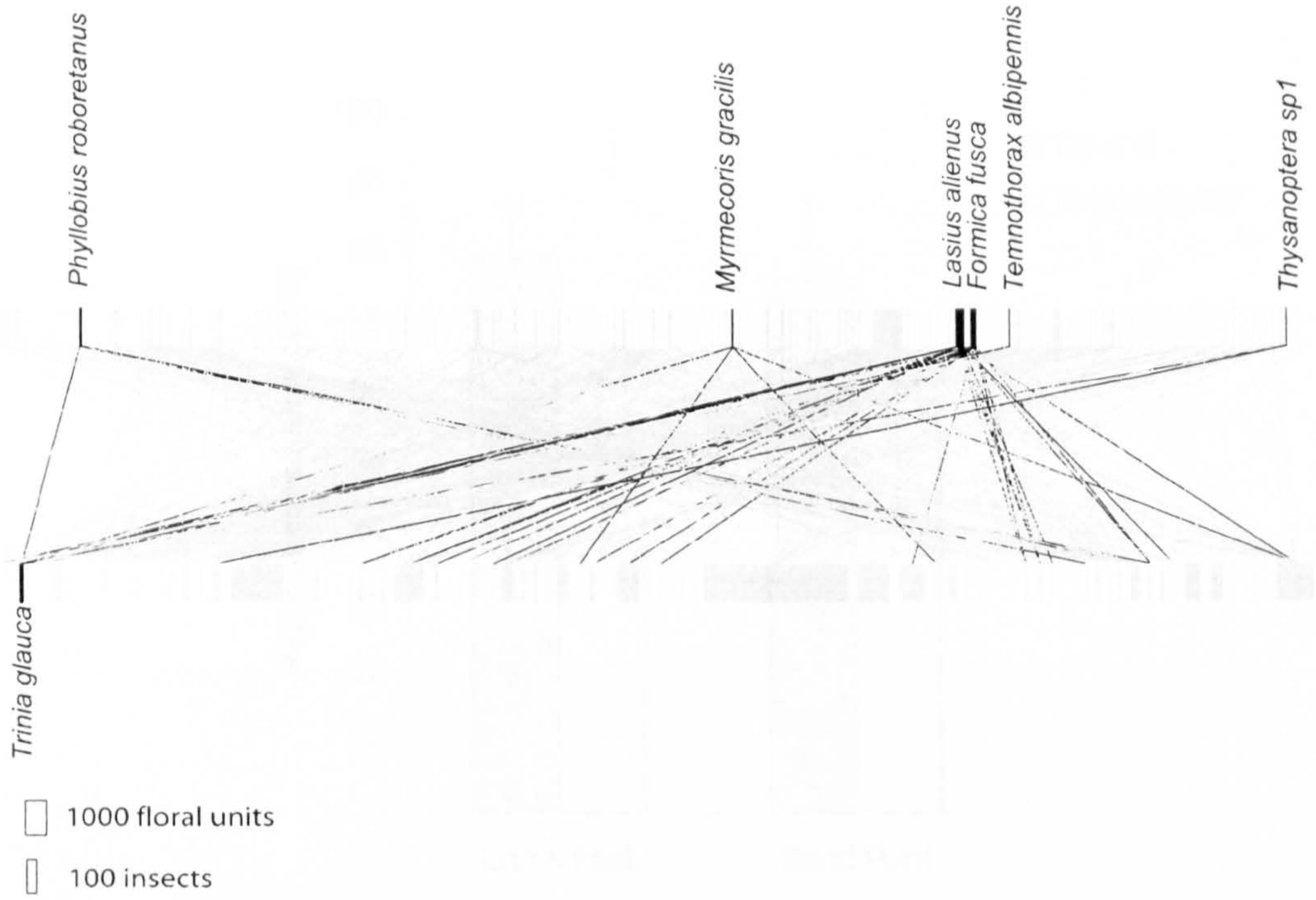

Figure 2.1 Flower visitation web for plant community at Avon Gorge. Each species of plant and insect is represented by a rectangle. A list of plant and visitor species is provided in supplementary material (Tables S2.1-2). The widths of the rectangles represent overall species abundance at the field site and the size of the lines connecting them represents the frequency of interaction in the study area. All *Trinia glauca's* interactions are shown in black. The scale bar represents number of flower units present in the study area and the number of insects present in the study area.

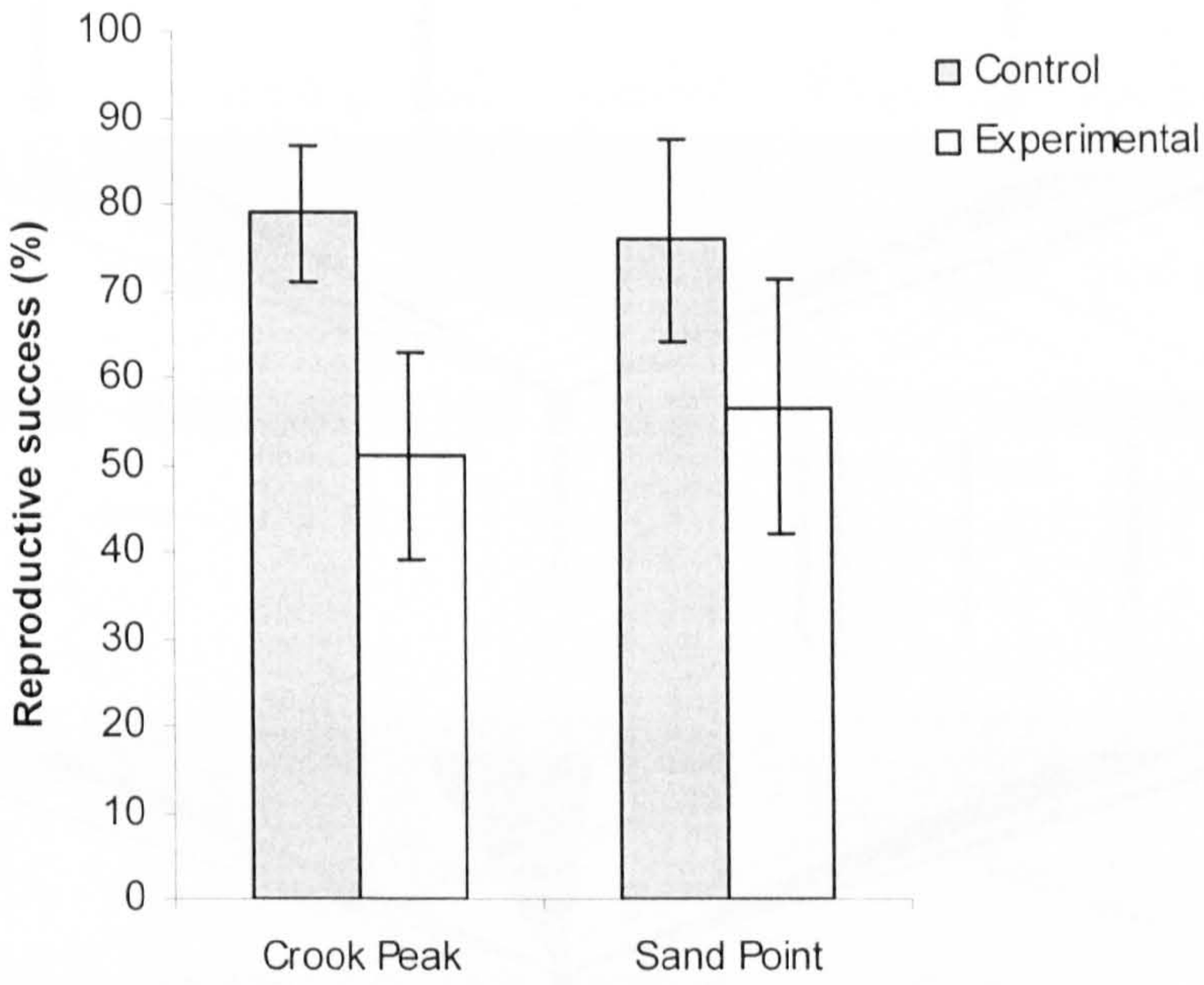

Figure 2.2 Average reproductive success (% of developed seeds) in Control and Experimental (ant-excluded) female plants of *T. glauca* at Crook Peak and Sand Point. Error bars represent the standard deviation.

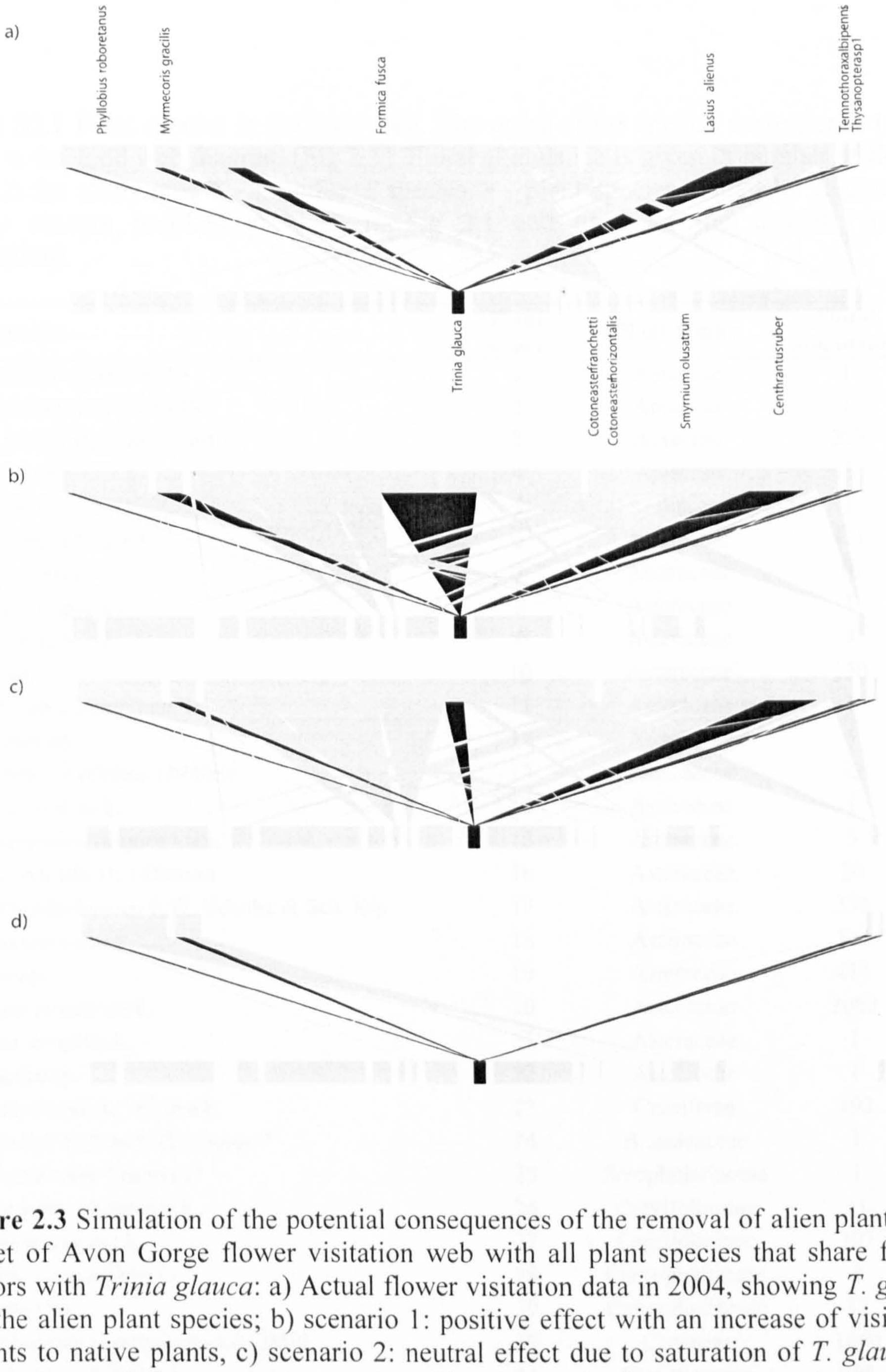

Figure 2.3 Simulation of the potential consequences of the removal of alien plants in a subset of Avon Gorge flower visitation web with all plant species that share flower visitors with *Trinia glauca*: a) Actual flower visitation data in 2004, showing *T. glauca* and the alien plant species; b) scenario 1: positive effect with an increase of visitation by ants to native plants, c) scenario 2: neutral effect due to saturation of *T. glauca* as food source and d) scenario 3: negative effect due to decline of ants population. Each species of plant and insect is represented by a rectangle, with the aliens removed from figures 2.3b-d. All *T. glauca's* interactions are shown in black. For explanation of the figure see Fig 2.1.

Supplementary material

Table S2.1 Plant species in the food web. The order of the species corresponds to the order in the food web diagram (Fig 2.1). Floral abundance is given in number of flower units in the study area * - introduced species. • - plant species visited by *T. glauca*'s flower visitors (marked in black in Fig 2.1 and used for the species removal simulation).

Plant species	Plant order	Plant family	Floral abundance
Smyrnium olusatrum L.* •	1	Apiaceae	1
Torilis japonica (Houtt.) DC	2	Apiaceae	1
Trinia glauca (L.) Dum mort •	3	Apiaceae	203
Apiaceae sp.	4	Apiaceae	1
Hedera helix L.	5	Araliaceae	1
Achillea millefolium L.	6	Asteraceae	411
Bellis perenis L.	7	Asteraceae	16
Carduus crispus L.	8	Asteraceae	1
Carlina vulgaris L.	9	Asteraceae	4
Crepis sp.	10	Asteraceae	150
Eupatorium cannabinum L.	11	Asteraceae	1
Hieracium sp.	12	Asteraceae	5
Inula conyzae (Griess.) Meikle	13	Asteraceae	32
Lactuca serriola L.	14	Asteraceae	1
Leucanthemum vulgare Lam.	15	Asteraceae	5
Mycelis muralis (L.) Dumort	16	Asteraceae	80
Pilosella officinarum F.W. Schultz & Sch. Bip	17	Asteraceae	331
Senecio jacobaea L.	18	Asteraceae	144
Senecio sp.	19	Asteraceae	411
Solidago virgaurea L.	20	Asteraceae	3080
Sonchus arvensis L.	21	Asteraceae	1
Asteraceae sp.	22	Asteraceae	1
Erophila verna (L.) Chevall.	23	Cruciferae	192
Sisymbrium officinale (L.) Scop. *	24	Brassicaceae	1
Buddleja davidii Franchet*	25	Scrophulariaceae	1
Lonicera peryclymenum L.	26	Caprifoliaceae	11
Viburnum lantana L.	27	Caprifoliaceae	107
Arenaria serpyllifolia L.	28	Caryophyllaceae	1
Cerastium sp.	29	Caryophyllaceae	37
Helianthemum nummularium (L.)Mill.	30	Cistaceae	1660
Hypericum perforatum L.	31	Hypericaceae	288
Hypericum sp.	32	Hypericaceae	11
Cornus sanguinea L.	33	Cornaceae	96
Sedum acre L.	34	Crassulaceae	16
Sedum album L. *	35	Crassulaceae	1

Tamus communis L.	36	Dioscoreaceae	1
Scabiosa columbaria L.	37	Dipsacaceae	539
Anthyllis vulneraria L.	38	Fabaceae	5
Hippocrepis comosa L.	39	Fabaceae	139
Lotus corniculatus L.	40	Fabaceae	75
Medicago lupina L.	41	Fabaceae	641
Trifolium pratense L.	42	Fabaceae	1
Ulex europaeus L.	43	Fabaceae	326
Blackstonia perfoliata (L.) Hudson	44	Gentianaceae	1
Geranium robertianum L.	45	Geraniaceae	1297
Geranium rotundifolia L.	46	Geraniaceae	21
Clinopodium acinos (L.) Kuntze	47	Lamiaceae	48
Clinopodium ascendens (Jord.) Samp.	48	Lamiaceae	5
Clinopodium vulgare L.	49	Lamiaceae	43
Euphrasia nemorosa (Pers.) Wallr.	50	Scrophulariacea	16
Origanum vulgare L.	51	Lamiaceae	8434
Teucrium scorodonia L.	52	Lamiaceae	1810
Thymus polytrichus A. Kerner ex Borbás	53	Lamiaceae	1468
Allium sphaerocephalon L.	54	Alliaceae	48
Ligustrum vulgare L.	55	Oleaceae	561
Orobanche hederae Duby	56	Orobanchaceae	1
Plantago lanceolata L.	57	Plantaginaceae	107
Clematis vitalba L.	58	Ranunculaceae	107
Ranunculus sp1	59	Ranunculaceae	1
Ranunculus sp2	60	Ranunculaceae	1
Cotoneaster franchetti Bois * •	61	Rosaceae	214
Cotoneaster horizontalis Decne * •	62	Rosaceae	160
Crataegus monogyna Jacq.	63	Rosaceae	85
Geum urbanum L.	64	Rosaceae	1
Potentilla neumanniana Rchb.	65	Rosaceae	43
Potentilla sterilis (L.) Garcke	66	Rosaceae	37
Rosa arvensis Huds.	67	Rosaceae	69
Rosa canina L.	68	Rosaceae	5
Rubus fruticosus L.	69	Rosaceae	64
Sanguisorba minor Scop.	70	Rosaceae	454
Sorbus aria (L.) Crantz	72	Rosaceae	80
Gallium aparine L.	73	Rubiaceae	144
Gallium verum L.	74	Rubiaceae	1
Rubia peregrina L.	75	Rubiaceae	929
Melampyrum pratense L.	76	Scrophulariaceae	742
Verbascum thapsus L.	77	Scrophulariaceae	1
Veronica sp.	78	Scrophulariaceae	5
Solanum dulcamara L.	79	Solanaceae	21
Urtica dioica L.	80	Urticaceae	1
Centranthus ruber (L.) DC. * •	81	Valerianaceae	2301

Table S2.2 Visitor species in the food web. The order of the species corresponds to the order in the food web diagram (Fig 2.1). • - Visitor species included in the complex system approach.

Visitor species	Order	Visitor family	Plant resources
Cteniopus sulphurous L.	1	Coleoptera: Tenebrionidae	*A. sphaerocephalon* *C. ruber* *H. perfuratum* *L. vulgare*
Isomira murina L.	2	Coleoptera: Tenebrionidae	*C. monogyna* *S. olusatrum* *V. lantana*
Apion ulicis Forster	3	Coleoptera: Curculionidae	*U. europaeus*
Byturus tomentosus Fabricius	4	Coleoptera: Byturidae	*C. monogyna* *R. fruticosus*
Cantharis decipiens Baudi	5	Coleoptera: Cantharidae	*C. monogyna*
Rhagonycha fulva Scopoli	6	Coleoptera: Cantharidae	*E. cannabinum*
Grammoptera ruficornis Fabricius	7	Coleoptera: Cerambycidae	*C. monogyna*
Strangalia maculata Poda	8	Coleoptera: Cerambycidae	*R. fruticosus*
Aphthona herbigrada Curtis	9	Coleoptera: Chrysomelidae	*H. nunmularium*
Anthonomus rubi Herbst	10	Coleoptera: Curculionidae	*R. fruticosus* *S. dulcamara*
Centhorhynchus sp.	11	Coleoptera: Curculionidae	*S. officinale*
Phyllobius roboretanus Gredler •	12	Coleoptera: Curculionidae	*P. sterilis* *S. minor* *S. aria* ***T. glauca***
Phyllobius viridiaeris Laicharting	13	Coleoptera: Curculionidae	*T. pratense*
Curculionidae sp1	14	Coleoptera: Curculionidae	*L. corniculatus*
Agrypnus murinus L.	15	Coleoptera: Elateridae	*S. olusatrum*
Mordellistena neuwaldeggiana Panzer	16	Coleoptera: Mordellidae	*R. fruticosus*
Meligethes aeneus Fabricius	17	Coleoptera: Nitidulidae	*C. monogyna* *Crepis* sp. *G. robertianum* *G. urbanum* *M. muralis* *O. vulgare* *R. arvensis* *R. fruticosus* *S. arvensis*
Meligethes atratus Oliver	18	Coleoptera: Nitidulidae	*G. robertianum*
Meligethes flavimanus Stephens	19	Coleoptera: Nitidulidae	*G. robertianum* *R. canina*
Meligethes obscurus Erichson	20	Coleoptera: Nitidulidae	*C. ruber* *Crepis* sp. *Hieracium* sp. *P. sterilis*
Meligethes solidus Kugelann	21	Coleoptera: Nitidulidae	*S. aria* *T. scorodonia* Asteraceae sp. *B. perenis* *C. ruber* *Crepis* sp. *G. robertianum* *H. nunmularium* *Hieracium* sp. *H. comosa*

Meligethes solidus Kugelann (cont)			*P. officinarum* *Ranunculus* sp1 *R. fruticosus* *S. columbaria* *S. olusatrum* *V. thapsus*
Oedemera lurida Marsham	22	Coleoptera: Oedemeridae	*A. millefolium* *C. ruber* *Crepis* sp. *H. nunmularium* *H. comosa* *O. vulgare* *S. minor* *Senecio* sp.
Oedemera nobilis Scopoli	23	Coleoptera: Oedemeridae	*A. millefolium* *C. ruber* *H. nunmularium* *L. vulgare* *P. officinarum*
Cetonia aurata L.	24	Coleoptera: Scarabaeidae	*C. monogyna*
Anaspis frontalis L.	25	Coleoptera: Scraptiidae	*R. arvensis*
Anaspis pulicaria Costa	26	Coleoptera: Scraptiidae	*C. vitalba* *C. sanguinea* *Hieracium* sp. *L. vulgare* *R. canina* *R. fruticosus* *U. europaeus* *V.lantana*
Eusphalerum luteum Marsham	27	Coleoptera: Staphylinidae	*E. cannabinum* *R. fruticosus*
Anthomyia liturata Robineau-Desvoidy	28	Diptera: Anthomyiidae	*S. virgaurea*
Delia sp.	29	Diptera: Anthomyiidae	*H. nunmularium* *L. serriola* *S. columbaria* *S. olusatrum*
Heterostylodes nominabilis Collin	30	Diptera: Anthomyiidae	*Crepis* sp. *Hieracium* sp. *P. officinarum* *Ranunculus* sp1
Pegoplata aestiva Meigen	31	Diptera: Anthomyiidae	*H. nunmularium* *H. perfuratum* *Senecio* sp. *S. arvensis*
Anthomyiidae sp1	32	Diptera: Anthomyiidae	*S. officinale*
Anthomyiidae sp2	33	Diptera: Anthomyiidae	*H. perfuratum*
Bombylius major L.	34	Diptera: Bombyliidae	*C. ruber* *G. robertianum*
Lucilia illustris Meigen	35	Diptera: Calliphoridae	*S. columbaria* *S. jacobaea* *Senecio* sp.
Pollenia rudis Fabricius	36	Diptera: Calliphoridae	*S. virgaurea*
Orthocladiinae cf. sp.	37	Diptera: Chironomidae	*C. vitalba*
Conioscinella sp.	38	Diptera: Chloropidae	*M. lupina*
Lasiambia palposa Fallén	39	Diptera: Chloropidae	*L. vulgare* *Senecio* sp. *T. polytrichus*
Trachysiphonella sp1	40	Diptera: Chloropidae	*G. verum*

Trachysiphonella sp2	41	Diptera: Chloropidae	*R. canina*
Chloropidae sp1	42	Diptera: Chloropidae	*T. polytrichus*
Conops quadrifasciatus De Geer	43	Diptera: Conopidae	*Senecio* sp.
Discomyza incurva Fallén	44	Diptera: Ephydridae	Apiaceae sp.
			G. verum
Platypalpus nigritarsis Fallén cf.	45	Diptera: Hybotidae	*R. arvensis*
Peplomyza litura Meigen	46	Diptera: Lauxaniidae	*R. fruticosus*
Lauxaniidae sp1	47	Diptera: Lauxaniidae	*C. vitalba*
Neomyia cornicina Fabricius	48	Diptera: Muscidae	*S. virgaurea*
Muscidae sp1	49	Diptera: Muscidae	*U. europaeus*
Megaselia sp.	50	Diptera: Phoridae	*C. monogyna*
Eudorylas sp.	51	Diptera: Pipunculidae	*C. vulgare*
Rhinophora lepida Meigen	52	Diptera: Rhinophoridae	*A. millefolium*
			G. rotundifolia
			S. officinale
Brachicoma devia Fallen	53	Diptera: Sarcophagidae	*Senecio* sp.
Heteronychia haemorrhoa Meigen	54	Diptera: Sarcophagidae	*A. millefolium*
Sarcophaga carnaria L.	55	Diptera: Sarcophagidae	*T. japonica*
Sarcophaga nigriventris Meigen	56	Diptera: Sarcophagidae	*E. cannabinum*
			S. jacobaea
			S. virgaurea
			T. japonica
Sarcophaga subvicina Rohdendorf	57	Diptera: Sarcophagidae	*S. virgaurea*
Sarcophaga spp.	58	Diptera: Sarcophagidae	*H. helix*
			S. olusatrum
Sciaridae Gen1 sp.	59	Diptera: Sciaridae	*Senecio* sp.
Sciaridae Gen2 sp.	60	Diptera: Sciaridae	*H. helix*
Sepsis cf. *fulgens* Meigen	61	Diptera: Sepsidae	*C. vulgare*
Sepsis cynipsea L.	62	Diptera: Sepsidae	*C. vitalba*
Baccha sp.	63	Diptera: Syrphidae	*M. muralis*
Cheilosia soror Zetterstedt	64	Diptera: Syrphidae	*T. japonica*
Dasysyrphus tricinctus Fallén	65	Diptera: Syrphidae	*P. officinarum*
Epistrophe grossularia Meigen	66	Diptera: Syrphidae	*C. ruber*
			S. columbaria
			S. arvensis
			T. scorodonia
			T. japonica
Episyrphus balteatus DeGeer	67	Diptera: Syrphidae	*C. ruber*
			C. vitalba
			Crepis sp.
			G. verum
			G. robertianum
			H. nunmularium
			H. perfuratum
			L. serriola
			M. muralis
			O. vulgare
			P. officinarum
			R. fruticosus
			S. columbaria
			S. dulcamara
			S. virgaurea
Eristalis arbustorum L.	68	Diptera: Syrphidae	*A. millefolium*
			S. columbaria
			Senecio sp.
Eristalis interruptus Poda	69	Diptera: Syrphidae	*S. virgaurea*
Eristalis intricarius L.	70	Diptera: Syrphidae	*R. fruticosus*
Eristalis pertinax Scopoli	71	Diptera: Syrphidae	*S. columbaria*
Eristalis tenax L.	72	Diptera: Syrphidae	*Crepis* sp.

Eristalis tenax L. (cont)			*E. cannabinum*
			Hieracium sp.
			S. columbaria
			S. virgaurea
Eupeodes luniger Meigen	73	Diptera: Syrphidae	*L. serriola*
			S. columbaria
			S. jacobaea
Ferdinandea cuprea Scopoli	74	Diptera: Syrphidae	*Crepis* sp.
			Hieracium sp.
			M. muralis
Helophilus pendulus L.	75	Diptera: Syrphidae	Apiaceae sp.
			C. vitalba
			C. ascendens
			H. helix
			S. columbaria
			S. virgaurea
Melangyna umbellatarum Fabricius	76	Diptera: Syrphidae	*C. franchetti*
			H. nunmularium
			O. vulgare
Melanostoma mellinum L.	77	Diptera: Syrphidae	*Crepis* sp.
			H. nunmularium
			H. nunmularium
			L. serriola
			P. lanceolata
			Senecio sp.
Melanostoma scalare Fabricius	78	Diptera: Syrphidae	*Crepis* sp.
			G. robertianum
			H. nunmularium
			H. perfuratum
			L. serriola
			M. muralis
Meliscaeva auricollis Meigen	79	Diptera: Syrphidae	*C. ruber*
			R. arvensis
			R. fruticosus
Meliscaeva cinctella Zetterstedt	80	Diptera: Syrphidae	*C. vitalba*
Metasyrphus cf. *latilunulatus* Collin	81	Diptera: Syrphidae	*R. canina*
Metasyrphus corollae Fabricius	82	Diptera: Syrphidae	*Crepis* sp.
Metasyrphus latifasciatus Macquart	83	Diptera: Syrphidae	*S. virgaurea*
Metasyrphus luniger Meigen	84	Diptera: Syrphidae	*L. serriola*
Myathropa florea L.	85	Diptera: Syrphidae	*C. ruber*
			H. helix
Paragus haemorrhous Meigen	86	Diptera: Syrphidae	*A. millefolium*
			C. ruber
			G. verum
			H. nunmularium
			L. vulgare
			S. virgaurea
			T. polytrichus
			T. japonica
Platycheirus albimanus Fabricius	87	Diptera: Syrphidae	*C. vitalba*
			G. robertianum
			H. nunmularium
			Hieracium sp.
			H. perfuratum
			M. lupina
			R. fruticosus
			S. jacobaea

Platycheirus albimanus Fabricius (cont)			*Senecio* sp.
			S. virgaurea
Platycheirus amplus Curran cf.	88	Diptera: Syrphidae	*G. robertianum*
Platycheirus clypeatus Meigen	89	Diptera: Syrphidae	*M. muralis*
			S. minor
Platycheirus scutatus Meigen	90	Diptera: Syrphidae	*H. perfuratum*
			M. muralis
Sphaerophoria scripta L.	91	Diptera: Syrphidae	*A. sphaerocephalon*
			C. vitalba
			H. nunmularium
			H. perfuratum
			L. serriola
			P. officinarum
			Senecio sp.
			S. officinale
			S. virgaurea
			S. virgaurea
			T. japonica
Syritta pipiens L.	92	Diptera: Syrphidae	*A. millefolium*
			E. cannabinum
			L. serriola
Syrphus ribesii L.	93	Diptera: Syrphidae	*C. ruber*
			C. vitalba
			Crepis sp.
			E. cannabinum
			H. helix
			H. nunmularium
			Hieracium sp.
			L. serriola
			P. officinarum
			Ranunculus sp2
			R. fruticosus
			S. columbaria
			Senecio sp.
			S. arvensis
			T. japonica
Syrphus torvus Osten Sacken	94	Diptera: Syrphidae	*L. vulgare*
			S. columbaria
Syrphus vitripennis Meigen	95	Diptera: Syrphidae	*C. vitalba*
			Hieracium sp.
			Senecio sp.
Volucella pellucens L.	96	Diptera: Syrphidae	*L. vulgare*
Dexiosoma caninum Fabricius	97	Diptera: Fabricius	*R. fruticosus*
Eriothrix rufomaculata DeGeer	98	Diptera: Tachinidae	*A. millefolium*
			C. vitalba
			E. cannabinum
			H. nunmularium
			Senecio sp.
Phasia pusilla Meigen	99	Diptera: Tachinidae	*A. millefolium*
Solieria pacifica Meigen	100	Diptera: Tachinidae	*A. millefolium*
			Apiaceae sp.
Tachina fera L.	101	Diptera: Tachinidae	*S. virgaurea*
Myrmecoris gracilis R.F.Sahlberg •	102	Heteroptera: Miridae	*A. sphaerocephalon*
			C. ruber
			T. pratense
			T. glauca
Heteroptera sp1	103	Heteroptera	*U. europaeus*
Heteroptera sp2	104	Heteroptera	*A. millefolium*

Heteroptera sp3	105	Heteroptera	*S. columbaria*
Heteroptera sp4	106	Heteroptera	*A. millefolium*
Heteroptera sp5	107	Heteroptera	*H. nunmularium* *R. fruticosus*
Heteroptera sp6	108	Heteroptera	*L. vulgare*
Heteroptera sp7	109	Heteroptera	*A. millefolium* *C. vitalba* *G. robertianum* *O. vulgare*
Heteroptera sp8	110	Heteroptera	*O. vulgare* *Senecio* sp.
Heteroptera sp9	111	Heteroptera	*E. cannabinum* *R. fruticosus*
Homoptera sp1	112	Homoptera	*C. ruber*
Homoptera sp2	113	Homoptera	*C. ruber*
Homoptera sp3	114	Homoptera	*C. ruber*
Andrena bicolor Fabricius	115	Hymenoptera: Andrenidae	*G. robertianum*
Andrena minutula Kirby	116	Hymenoptera: Andrenidae	*A. millefolium*
Andrena sp3	117	Hymenoptera: Andrenidae	*P. officinarum*
Apis mellifera L.	118	Hymenoptera: Apidae	*C. vitalba* *R. fruticosus*
Bombus hortorum L.	119	Hymenoptera: Apidae	*C. ruber* *L. vulgare* *R. arvensis* *T. scorodonia*
Bombus lapidarius L.	120	Hymenoptera: Apidae	*A. sphaerocephalon* *C. franchetti* *Crepis* sp. *H. nunmularium* *H. camosa* *L. corniculatus* *R. fruticosus* *S. columbaria* *Senecio* sp.
Bombus lucorum L.	121	Hymenoptera: Apidae	*L. vulgare* *O. vulgare*
Bombus pascuorum Scopoli	122	Hymenoptera: Apidae	*A. sphaerocephalon* Apiaceae sp. *B. davidii* *C. vulgaris* *C. ruber* *C. vitalba* *C. acinos* *C. ascendens* *C. franchetti* *Crepis* sp. *G. robertianum* *G. rotundifolia* *H. nunmularium* *Hieracium* sp. *H. camosa* *H. perfuratum* *L. corniculatus* *M. pratense* *O. vulgare* *P. officinarum* *Ranunculus* sp1 *R. arvensis* *R. fruticosus*

Bombus pascuorum Scopoli (cont)			*S. columbaria* *S. dulcamara* *S. virgaurea* *S. arvensis* *T. scorodonia* *T. japonica* *U. europaeus*
Bombus pratorum L.	123	Hymenoptera: Apidae	*C. franchetti* *G. robertianum* *L. vulgare* *R. arvensis* *R. canina*
Bombus terrestris L.	124	Hymenoptera: Apidae	*C. ruber* *C. franchetti* *Crepis* sp. *R. fruticosus* *Senecio* sp.
Bombus vestalis Geoffroy	125	Hymenoptera: Apidae	*O. vulgare* *S. virgaurea*
Ceratina cyanea Kirby	126	Hymenoptera: Apidae	*H. nunmularium* *P. officinarum*
Arge pagana Panzer	127	Hymenoptera: Argidae	Apiaceae sp.
Hylaeus communis Nylander	128	Hymenoptera: Colletidae	*C. vitalba* *Senecio* sp.
Hylaeus hyalinatus Smith	129	Hymenoptera: Colletidae	*A. millefolium*
Formica fusca L. •	130	Hymenoptera: Formicidae	Apiaceae sp. *C. ruber* *C. sanguinea* *C. franchetti* *C. horizontalis* *C. monogyna* *G. verum* *H. helix* *H. nunmularium* *H. perfuratum* *S. olusatrum* *S. aria* *T. communis* *T. japonica* ***T. glauca*** *V. lantana*
Lasius alienus Foerster •	131	Hymenoptera: Formicidae	*C. acinos* *C. franchetti* *C. horizontalis* *G. robertianum* *H. nunmularium* *S. columbaria* *S. aria* *T. polytrichus* ***T. glauca*** *U. europaeus* *V. lantana*
Lasius niger L.	132	Hymenoptera: Formicidae	*C. acinos* *C. sanguinea*
Myrmica lobicornis Nylander	133	Hymenoptera: Formicidae	*G. verum*
Myrmica ruginoides L.	134	Hymenoptera: Formicidae	*P. sterilis*
Myrmica sp3	135	Hymenoptera: Formicidae	*R. fruticosus* *S. columbaria*
Temnothorax albivennis Curtis •	136	Hymenoptera: Formicidae	***T. glauca***

Halictus tumulorum L.	137	Hymenoptera: Halictidae	*A. millefolium* *A. sphaerocephalon* *Crepis* sp. *O. vulgare* *R. fruticosus* *Senecio* sp. *S. officinale* *T. japonica* *V. thapsus*
Lasioglossum albipes Fabricius	138	Hymenoptera: Halictidae	*E. cannabinum*
Lasioglossum calceatum Scopoli	139	Hymenoptera: Halictidae	*A. sphaerocephalon* *Crepis* sp. *Hieracium* sp. *Senecio* sp. *S. virgaurea*
Lasioglossum fulvicorne Kirby	140	Hymenoptera: Halictidae	*C. ruber* *S. virgaurea*
Lasioglossum leucopus Kirby	141	Hymenoptera: Halictidae	*A. sphaerocephalon* *C. vitalba* *T. japonica*
Lasioglossum morio Fabricius	142	Hymenoptera: Halictidae	*A. sphaerocephalon* *C. vitalba* *C. acinos* *C. ascendens* *Crepis* sp. *H. helix* *Hieracium* sp. *L. corniculatus* *M. lupina* *O. vulgare* *P. officinarum* *R. arvensis* *S. columbaria* *Senecio* sp. *S. virgaurea* *T. japonica*
Lasioglossum parvulum Schenck	143	Hymenoptera: Halictidae	*G. robertianum*
Lasioglossum quadrinotatum Schenck	144	Hymenoptera: Halictidae	*S. virgaurea*
Lasioglossum smeathmanellum Kirby	145	Hymenoptera: Halictidae	*A. sphaerocephalon* *C. vitalba* *Hieracium* sp. *L. serriola* *P. officinarum* *Ranunculus* sp1 *S. virgaurea*
Lasioglossum villosulum Kirby	146	Hymenoptera: Halictidae	*C. ruber* *Crepis* sp. *Hieracium* sp. *O. vulgare* *P. officinarum* *S. arvensis*
Sphecodes niger Hagen	147	Hymenoptera: Halictidae	*A. millefolium* *Crepis* sp.
Hoplitis claviventris Thomson	148	Hymenoptera: Megachilidae	*S. columbaria*
Hoplitis spinulosa Kirby	149	Hymenoptera: Megachilidae	*Crepis* sp. *Hieracium* sp. *O. vulgare* *P. officinarum* *Senecio* sp.

Hymenoptera sp1	150	Hymenoptera	*A. millefolium*
Hymenoptera sp2	151	Hymenoptera	*S. olusatrum*
Hymenoptera sp3	152	Hymenoptera	Apiaceae sp.
Hymenoptera sp4	153	Hymenoptera	*C. ascendens*
Hymenoptera sp5	154	Hymenoptera	*H. helix*
Hymenoptera sp6	155	Hymenoptera	*H. nunmularium*
Hymenoptera sp7	156	Hymenoptera	*Hieracium* sp.
Hymenoptera sp8	157	Hymenoptera	*M. lupina*
Hymenoptera sp9	158	Hymenoptera	*P. lanceolata*
Hymenoptera sp10	159	Hymenoptera	*S. columbaria*
Hymenoptera sp11	160	Hymenoptera	*Senecio* sp.
Ectemnius continuus Fabricius	161	Hymenoptera: Crabronidae	*T. japonica*
Tenthredo sp.	162	Hymenoptera: Tenthredinidae	Apiaceae sp.
Vespula vulgaris L.	163	Hymenoptera : Vespidae	*G. verum*
			H. helix
Ochlodes venata Bremer & Grey	164	Lepidoptera: Hesperiidae	*T. scorodonia*
Polyommatus icarus Rottemburg	165	Lepidoptera: Lycaenidae	*I. conyzae*
			L. corniculatus
			O. vulgare
			P. officinarum
			S. columbaria
			S. virgaurea
Lepidoptera sp1	166	Lepidoptera	*S. columbaria*
Lepidoptera sp2	167	Lepidoptera	*L. vulgare*
Lepidoptera sp3	168	Lepidoptera	*U. europaeus*
Aglais urticae L.	169	Lepidoptera: Nymphalidae	*C. ruber*
			S. columbaria
Gonepteryx rhamni L.	170	Lepidoptera: Pieridae	*O. vulgare*
Pieris napi L.	171	Lepidoptera: Pieridae	*C. ruber*
			G. robertianum
			H. nunmularium
			S. columbaria
Aphantopus hyperantus L.	172	Lepidoptera: Nymphalidae	*T. scorodonia*
Melanargia galathea L.	173	Lepidoptera: Nymphalidae	*C. ruber*
			O. vulgare
			S. columbaria
Pyronia tithonus L.	174	Lepidoptera: Satyridae	*C. ruber*
			O. vulgare
			S. columbaria
Thysanoptera sp1 •	175	Thysanoptera	*Senecio* sp.
			T. glauca
Thysanoptera sp2	176	Thysanoptera	*Senecio* sp.
Thysanoptera sp3	177	Thysanoptera	*Ranunculus* sp1
Thysanoptera sp4	178	Thysanoptera	*Ranunculus* sp1
Thysanoptera sp5	179	Thysanoptera	*Crepis* sp.

Chapter 3

DIET BREADTH DETERMINES HOW IMPACTS OF INVASIVE PLANTS ARE PROPAGATED THROUGH FOOD WEBS

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Summary: Invasive plants are a major cause of ecosystem alteration worldwide. While their impacts on native plant communities are widely reported, there are few data on how such impacts propagate through food webs and affect species at higher trophic levels. In this study, I use a quantitative food web approach to evaluate the impacts of an invasive weed of UK heathlands, *Gaultheria shallon*, on plant-herbivore-parasitoid communities, determining specifically how diet breadth influences the propagation of such impacts.

The results of this work reveal that the weed *G. shallon* was an unsuitable food resource for native herbivores and that diet breadth of consumers affected how impacts of *G. shallon* propagate through the food web. *Gaultheria shallon* not only changed the abundance and species richness of herbivore and parasitoid insect communities but also changed the food web structure, which became dominated by generalist species and more uniform (higher evenness) as the abundance of the invasive plant increased in the study area. Therefore, evaluations of impacts based on native plants alone may considerably underestimate the overall impact of invasion on biodiversity. Considering higher trophic levels as well as the trophic diversity within levels is essential for a full evaluation of the consequences of disturbances.

When evaluating the predictability of the impacts at higher trophic levels, I found that both diet breath and the proportion of resources affected by the invasion are likely to have a key role in the regulation of the propagation of impacts through plant-herbivore-parasitoid food webs. Thus, information provided by quantitative food webs is important to improve predictive studies that evaluate consequences of species decline at a community level.

Key words: alien plants, trophic cascade, herbivory, diet breadth, quantitative food web.

Introduction

Invasive plants are seen as a serious threat to biodiversity worldwide (Vitousek *et al.* 1997), changing habitat by altering soil conditions (*e.g.* Feng *et al.* 2007; Isermann *et al.* 2007), altering disturbance regimes (*e.g.* D'Antonio & Vitousek 1992) and out-competing native plants (*e.g.* Weiss & Noble 1984a; Minchinton *et al.* 2006). As bottom-up trophic cascades are a common phenomenon in terrestrial systems (*e.g.* Baez *et al.* 2006; Price & Hunter 2005), the consequences of plant invasions could be far more extensive than those detected on plant communities, affecting species elsewhere in the trophic web, such as pollinators (*e.g.* Lopezaraiza-Mikel *et al.* 2007), as well as herbivores and parasitoids. However, most studies on impacts of invasive plants focus solely at plant level, disregarding terrestrial invertebrates (reviewed by Parker *et al.* 1999; more recent examples: Hulme & Bremner 2006; El-Keblawy & Al-Rawai 2007; Flory *et al.* 2007; Stinson *et al.* 2007). There are, however, some examples of studies that evaluate the impact of introduced plants on plant-pollinator systems at the population (Chittka & Schurkens 2001) or community level (Morales & Aizen 2006; Lopezaraiza-Mikel *et al.* 2007), revealing positive effects of invasion on the overall studied insect community. Plant-pollinator systems can contain several highly generalist species (*e.g.* Memmott 1999; Olesen & Jordano 2002). As generalist and specialist guilds can react to disturbances in very distinct ways (Rand & Tscharntke 2007) evaluating impacts of plant invasion on communities dominated by specialist species, such as herbivores, may reveal different results to those obtained for pollinators.

Being able to predict the impacts of invasive species on overall communities is a highly desirable goal as it could guide the development of better management strategies (Buckley *et al.* 2006). To predict how food webs change after a given disruption (such as plant invasion) an understanding of the mechanisms that influence the strength of impacts is needed (Chase 1996). As specialist guilds may have higher susceptibility to habitat changes than generalists (*e.g.* Zabel & Tscharntke 1998; Rand & Tscharntke 2007), diet breadth may thus have an important role in the propagation of impacts. Previous studies showed that highly connected food webs (*i.e.* food webs with several generalist species) are more robust to species extinction (Dunne *et al.* 2002). However,

it is not yet clear how diet breadth regulates the propagation of impacts through trophic levels (Fox 2007).

Heathlands in the UK are an anthropogenic habitat maintained by grazing (Webb 1986) that, despite having a very low diversity of plants, provide key refugia for a high number of rare and endangered species (Webb & Haskins 1980; Liley & Clarke 2003). The simplicity of the flora makes heathlands a good natural system in which to study trophic cascades as it is relatively straightforward to construct quantitative food webs in this habitat. Currently UK heathlands are threatened by recent invasions of introduced plants such as *Gaultheria shallon* Pursh (Ericaceae) and *Rhododendron ponticum* L. (Ericaceae) (Webb 1986). *Gaultheria shallon*, the subject of this study, is a densely growing perennial evergreen shrub native to North America heathlands that grows on a wide range of soil types and can be abundant in open habitats. This woody species was registered in the UK in the wild in 1914 (Preston *et al.* 2002) and is now one of the top 20 alien invasive species which threatens the UK's flora (Plantlife 2007). In this study I use a natural gradient of *G. shallon* invaded plots to evaluate how its impacts propagate through a plant-herbivore-parasitoid food web.

The objectives of this work are threefold: 1) To identify and quantify interactions among heathland plants, herbivores and parasitoids and evaluate how *G. shallon* integrates into the native community. Specialist enemies from introduced species are usually absent from the exotic range and generalist natural enemies are expected to have a greater impact on the native flora than on introduced species (Enemy Release Hypothesis, reviewed by Keane & Crawley 2002); therefore I predict that *G. shallon* will have both fewer generalist and specialist herbivores than native plants; 2) To determine how increases in *G. shallon* abundance affect the native food web. Species at higher trophic levels are thought to be at higher extinction risk than their resources (Cronin 2004); therefore, I predict that insect communities will be more affected than plants; 3) To test if the impact on consumer community can be predicted from the impact on resource abundance. More generalized consumers are more likely to include in their diet resource species that are not affected by the weed, which can be used as refugia; therefore I predict that: a) generalist consumers are less affected than specialist consumers; b) diet breadth influences how consumers respond to impacts on their resources.

Methods

Eight 1600 m^2 plots were selected in the Avon Heath Country Park, Dorset, UK, a Site of Special Scientific Interest composed of lowland heathlands (Grid references are provided in Table S3.1 in the supplementary material). Plots were selected to cover a gradient of *G. shallon* abundance (0.1%-80% of total plant cover) and separated by a minimum of 125m and a maximum of 945m. Plot dimensions varied slightly to avoid trails or forested areas, but the overall area was equal among plots. A total of seven surveys were carried out every 14-20 days on each plot from 27th June to 15th September 2005. In each survey, one transect (20m in length) was placed haphazardly per plot so that its entire length was within the plots and any overlap with previous transects was avoided. The percent coverage of each plant species present 1.5m either side of the transect was recorded. Leaf herbivores were collected by beating the plants along the transect. As these were mostly larval stages of insects, specimens were then individually reared until an adult herbivore or a parasitoid emerged or the specimen died (Henneman & Memmott 2001). Adult insect herbivores (*e.g.* grasshoppers or aphids) were not considered in this study. For herbivore species found at high abundance (more than 20 specimens per meter square) all individuals were counted but a sub-sample was collected for rearing. The total number of parasitoids reared from these species was then estimated by multiplying up the sub-sample. As some insect species overwinter as larvae or pupae the rearing period lasted until July 2006. Data for all seven transects were pooled (rearing data) or averaged (plant abundance) for each plot as appropriate. Herbivores and parasitoids were identified by taxonomists to the species level or morphotyped. Information from the literature was sufficient to distinguish parasitoids from hyperparasitoids. Data were used to construct quantitative plant-herbivore-parasitoid food webs for each plot, using software written in Mathematica™ (Memmott 1999) and used to answer the following three questions:

Q1: Is herbivory in G. shallon *lower than in native plants?*

Herbivory, calculated as the number of herbivore individuals per meter square of plant coverage, was determined in each plot for each of the abundant plant species (*i.e.* those present in all eight study plots), those being *G. shallon*, *Calluna vulgaris* (L.) Hull (heather), *Erica cinerea* L. (bell heather) and *Pteridium aquilinum* (L.) Kuhn (bracken) (for overall abundance data see Table S3.2). General Linear Mixed Models (GLMM) were used to test differences in herbivory between *G. shallon* and native plants, using plant origin (native *vs.* introduced) as a fixed factor and plot identity as a random factor. To normalize residuals, data on herbivory abundances were log transformed. Data were then analysed using Gaussian errors and *F* tests of changes in deviance.

Q2: How does G. shallon *impact on plant-herbivore-parasitoid food webs?*

General Linear Models (GLM) were first used to test the effect of the abundance of *G. shallon* on the abundance and species richness of plant and insect communities. I then considered the effect of *G. shallon* invasion on the overall food web structure (evenness and generalization). For each study plot, I calculated the species evenness of each trophic level, using the Shannon diversity index, and the evenness of species interactions, according to the methodology described by Tylianakis *et al.* (2007). Generalization, given by the proportion of specialist consumers on the community, of each consumer trophic levels, was also calculated. To normalize residuals, data on abundances and species richness were log transformed while evenness and proportion data were arcsin transformed. Data were then analysed using Gaussian errors and *F* tests of changes in deviance.

As only one plot had a *G. shallon* percentage cover greater than 50%, the leverage value of this plot was high (79.2% *G. shallon*), potentially having a strong influence on the models constructed. All study plots were located in a relatively constrained area subjected to equal management rules. Nevertheless, to explore the robustness of the results to the inclusion of this high abundance point, I repeated the analysis using just the seven plots where *G. shallon* abundance was lower than 50%. Results are reported for both the full and reduced data analyses.

Q3: Can the impact of* G. shallon *on consumers be inferred from its effect on resources?

To classify herbivores and parasitoids as generalists or specialists, I identified the resources of each consumer species combining the rearing data with published rearing records for herbivores (Porter 1997; NHM 2007a; Kimber 2007) and for parasitoids (Starke 1956; Sedivy 1967; Nixon 1974; Belshaw 1993; Lipa *et al.* 1993; Mills 1993; Shaw 1994; Karhu 1998; Bahena *et al.* 1999; Berry & Walker 2004; Kenis *et al.* 2005; Xuereb & Thiery 2006; Morales *et al.* 2007; Stenberg *et al.* 2007). This allowed a more precise identification of diet breadth, as information provided by rearing data is likely to be insufficient for rare species (*e.g.* if a generalist species was only collected once it would be wrongly considered a specialist). I defined specialists as species that feed exclusively on one family of resource species. Individual herbivores and parasitoids that could not be identified to species were not used in this analysis (see Tables S3.3-4). Species were then grouped in consumer subsets, according to trophic level and the type of resource used.

As not all resource species (here plants and herbivores) were affected by the abundance of *G. shallon* (see results) three general scenarios (see schematic representation in Fig 3.1) can be used to describe how consumers might respond to resource decline:

Scenario 1: Consumers are less affected by the invasive plant than their resources, increasing their density relative to their resources (*i.e.* the impact of *G. shallon* diminishes further up the food chain). Example: if only some of the resource species of a given consumer are affected by the alien plants, and assuming all resources are unsaturated (*i.e.* can support further insect foraging), the consumer may be able to adapt its diet and use the unaffected resources, that were already part of its diet, as refugia, thereby maintaining its abundance. In this case, impacts on the resource level overestimate impacts at the consumer level;

Scenario 2: Consumers are affected to the same extent as their resource species (*i.e.* 50% decrease in resources will lead to a 50% decrease of the consumer species), maintaining their density relative to their resources (*i.e.* the impact of *G. shallon* is constant going up the food chain). Example: the consumer is not able to adapt its

diet, but the decline in resources does not affect the reproductive success of the consumer on those resources that remain. In this case, impacts on the resource level are a good indicator of impacts at the consumer level;

Scenario 3: Consumer species are more affected than their resource species, decreasing their density relative to their resources (*i.e.* the impact of *G. shallon* is magnified going up the food chain). Example: the consumer is not able to adapt its diet, and the decline in resources does affect the reproductive success of the consumer (*i.e.* parametric threshold (Bascompte 2003) is achieved), leading to a decline more accentuated in consumers than in resources. In this case, impacts on the resource level underestimate impacts at the consumer level.

To understand how impacts on a given consumer subset are related to the impacts on their resources I first used GLM to test the effect of *G. shallon* abundance on consumer subsets and on their resources. To normalize residuals, data were log transformed and then analysed using Gaussian errors and *F* tests of changes in deviance. As described previously, I repeated the analysis using just the seven plots where *G. shallon* abundance was lower than 50%. Results are reported for both the full and reduced data analyses. Secondly, to test which consumer subsets fall in which scenarios, I paired each consumer subset with its resources (*e.g.* generalist herbivores *vs.* all native plants; specialist herbivores *vs.* all Ericaceae plants, *a priori* orthogonal contrasts). For each consumer-resource pair, I compared the strength of the weed's impact (given by the slope, β, of the regression models) on consumers and on their resources by testing for the significance of the interaction between the factorial variable trophic level (consumer vs. resource) and *G. shallon* abundance. This was done applying GLM and using an F-test on deviances with and without the interaction term. As abundance in plant and insect communities was measured in different units, abundance data for all three trophic levels was converted to percentages and log transformed to normalize residuals. However, if only one of the trophic levels is significantly affected (*e.g.* resources are affected but the consumer subset is not or *vice-versa*), testing if the slope of the effect of *G. shallon* on consumer and resources is significantly different is not required to define which scenario describes how the consumer subset responds to resource decline (see Fig 3.1). Thus, in those situations, significance of the interaction between trophic level and *G. shallon* was not tested. Although in some cases response

variables analyzed are inter-correlated (*e.g.* all native plants and Ericaceae plants), given that all hypotheses were formulated *a priori*, Bonferroni corrections were considered inappropriate as they would unjustifiably increase the probability of not detecting a real significant effect (Type II error) (Perneger 1998). All statistical analyses were performed with the software R (R Development Core Team 2007).

Results

A total of 3603 herbivores were collected from 12 plant species at the eight plots. Three orders of herbivores were collected: Coleoptera (one species) which comprised 87.4% of specimens, Lepidoptera (26 species) which comprised 11.0% of specimens and Hymenoptera (one species of Symphyta), which comprised 1.6% of specimens. A total of 2172 parasitoids were collected which consisted of 15 species of Hymenoptera and two species of Diptera. Finally, three specimens of a generalist obligatory endophagous hyperparasitoid species were reared from the lepidopteran herbivore *Gymnoscelis rufifasciata*. However, it was not possible to identify which parasitoid species were used as hosts and hence it was not included in the food webs dataset. Figure 3.2 illustrates the impact of 0.2%, 30.0% and 79.2% of *G. shallon* on heathland food webs (all eight food webs are provided in supplementary material, Figure S3.1), with detailed information on community species composition being provided in Table S3.2-4.

Q1: Is herbivory in G. shallon *lower than in native plants?*

The vast majority (99.8%) of the herbivore rearings came from native plants, and only 10 specimens (six species/morphospecies) were collected from *G. shallon*. When compared with the most common (*i.e.* abundant and present in all plots) native plant species in the study plots (*C. vulgaris*, *E. cinerea* and *P. aquilinum*), *G. shallon* was significantly less attacked by both generalist and specialist herbivores than the three native plant species (Generalist herbivores: $F_{23,1} = 4.8$, $P = 0.0396$, Average (*G. shallon*)

= 0.1 herbivores/m^2, Average (native plants) = 0.5 herbivores/m^2; Specialist herbivores: $F_{23,1}$ = 5.7, P = 0.0256, Average (*G. shallon*) = 0.0 herbivores/m^2, Average (native plants) = 3.2 herbivores/m^2).

Q2: How does* G. shallon *impact on plant-herbivore-parasitoid food webs?

Statistical results described in this section for plant, herbivore and parasitoid communities are presented in Table 3.1. Although a significant impact of *G. shallon* was detected on plant abundance, this was not seen in plots below 50% level of invasion (*i.e.* when considering just the seven plots). No significant effect of *G. shallon* was detected on plant species richness. *Gaultheria shallon* had a significant impact on the abundance and species richness of herbivore and parasitoid communities. Impacts on abundance were detectable below 50% invasion while impacts on species richness were not. Therefore, as expected insect communities were more affected than the plant community.

Concerning the impacts on the food web structure, *G. shallon* invasion significantly increased the evenness of all trophic levels and the generalization (proportion of generalist species) of both consumer trophic levels (herbivores and parasitoids). Evenness of species interactions (Tylianakis *et al.* 2007) in the overall food web also significantly increased ($F_{1,6}$ = 11.5; P = 0.01469). Significant increase of evenness and generalization were still detectable below 50% weed invasion.

Q3: Can the impact of* G. shallon *on consumers be inferred from its effect on resources?

Using the definition of a specialist being a species that feeds on resource species belonging to a single family and combining the rearing data with information from the literature, six groups of consumers were identified: 1) generalist herbivores (15 species); 2) herbivores specialist on Ericaceae plants (nine species); 3) generalist parasitoids (seven species); 4) Chrysomelidae specialist parasitoids (Coleoptera) (one species); 5) Noctuidae specialist parasitoids (Lepidoptera) (three species); 6) Geometridae specialist

parasitoids (Lepidoptera) (one species). Ericaceae specialists were never found on either of the two exotic Ericaceae (*G. shallon* and *R. ponticum*) present in the study area, so only native Ericaceae were considered as resources for this consumer subset.

As expected, *G. shallon* affected specialist and generalist subsets of communities differently, having significant impacts on the abundance of all specialist consumer subsets (herbivores and parasitoids), while no significant effect was detected for generalist consumer subsets (Fig 3.3). Concerning how impacts on consumers are related to impacts on their resources:

1) Generalist herbivores: Although the resources of generalist herbivores (all native plants) were negatively affected by *G. shallon* abundance, no significant effect was detected on the abundance of this consumer community (Table 3.2). Therefore, as described in Scenario 1, generalist herbivores were less affected than their resources, leading to an increase in their density relative to their resources.

2) Specialist herbivores: Both this consumer subset and their resources (Ericaceae plants) were significantly affected by *G. shallon* abundance (Table 3.2). Specialist herbivores were, however, significantly more affected than their resources ($F_{1,12}$ (interaction *G. shallon* x trophic level) = 51.4, P = 0.0035). Therefore, as described in Scenario 3, *G. shallon* abundance led to a reduction of specialist herbivore density relative to their resources.

3) Generalist parasitoids: Although the resources of generalist parasitoids were negatively affected by *G. shallon* abundance, no significant effect was detected on the abundance of this consumer subset (Table 3.2). Therefore, as described in Scenario 1, generalist parasitoids were less affected than their resources, leading to an increase in their density relatively to their resources.

4) Chrysomelidae specialist parasitoids 5) Noctuidae specialist parasitoids and 6) Geometridae specialist parasitoids: Each of these consumer subsets, as well as their resources, was significantly affected by *G. shallon* abundance (Table 3.2). However, all specialist consumer subsets had a decrease in proportion to their host-herbivores (Chrysomelidae specialist parasitoids: $F_{1,12}$ (interaction *G. shallon* x trophic level) = 19.2, P >0.05; Noctuidae specialist parasitoids: $F_{1,12}$ (interaction *G. shallon* x trophic level) = 9.8, P > 0.05; Geometridae specialist parasitoids: $F_{1,12}$ (interaction *G. shallon* x trophic level) = 7.4, P > 0.05). Therefore, as described in Scenario 2, specialist

parasitoids subsets were equally affected as their resources, maintaining their density relative to their resources.

Discussion

Although the impacts of invasive plants are a cause of considerable concern in conservation biology, most studies of invasive plants evaluate their effect only at the plant level, with little being known about the propagation of these impacts through trophic networks. The work presented here reveals that measuring impacts of the invasive *G. shallon* only at the plant level would underestimate the overall impact of the weed at the community level, as these were magnified in some consumer subsets leading to changes in the food web structure. In this section, I discuss the limitations of this study, and consider the results obtained in the light of the three questions posed, while highlighting the implications of my results for impact assessment studies using food webs as predictive tools.

Limitations

While quantitative food webs are a very useful tool when trying to understand the complexity of interactions at the community level, they do have some limitations. First, I could have had differential success in rearing parasitoid species, this influencing the estimates of species abundance. Additionally, I may have prevented some parasitoid attack by removing the herbivores from the field for rearing. Nevertheless, these limitations apply equally to all plots and consequently are unlikely to confound the sampling design.

Manipulative experiments which add invasive species in a controlled and replicated fashion would provide a better means of assessing impacts of invasive species (Siemann 1998). However, those experiments would put already threatened habitats, such as heathlands, at further risk. Therefore a 'natural experiment' approach utilising a gradient of disturbance, such as the one used in this study, is realistically the only

possible approach when working in protected habitats. Gradients in exotic plant abundance can be a consequence rather than a driver of biodiversity changes (Gurevitch & Padilla 2004; MacDougall & Turkington 2005; Thomas & Reid 2007). However, as all the study plots were located in a constrained area of a country park, being subjected to the same type of disturbances and management measures, there is no obvious reason, other than *G. shallon* invasion, for declines in native heathland plant species. While there are disadvantages, an advantage of this natural experiment approach also exists in that it allows system stabilization. As most experimental studies on trophic cascades only last one or a few resource/consumer generations (Borer *et al.* 2005), they do not capture long term processes, such as species extinction, and therefore are not adequate to study compositional change in communities (Leibold *et al.* 1997).

Q1: Is herbivory in* G. shallon *lower than in native plants?

The low number of herbivores on *G. shallon* in comparison to native plants is a result similar to those obtained in previous comparisons of native and exotic plants (*e.g.* Carpenter & Cappuccino 2005). However, as I defined specialists as species that feed on a family of resources, and as *G. shallon* belongs to the same family of the most common native heathland plants (Ericaceae), host switching could have occurred (Keane & Crawley 2002). However, it is clear that for UK heathland specialist herbivores and for the majority of generalist herbivores, *G. shallon* is not an alternative resource. This low level of herbivory may be due to the plant's defensive chemicals that are lethal to non-adapted native herbivores (Wiklund 1975; Chew 1977), which may also reduce pathogen attack and/or enable allelopathy (Carpenter & Cappuccino 2005).

Q2: How does* G. shallon *impact on plant-herbivore-parasitoid food webs?

The importance of bottom-up effects in ecosystems is well recognized. For example, Balciunas and Lawler (1995) found that species of protists were more affected by changes in the abundance of their resource bacteria than by changes in the abundance of

its predator species; while Price and Hunter (2005) found that oviposition of a stem-gall forming fly increased due to changes in the quality of its resources. The work here presented provides a further example, and is the first to use a food web approach to examine impacts of an invasive plant on herbivores and parasitoids. Further impacts were detected in the food web structure, with communities becoming dominated by generalist species and more homogeneous (higher evenness) with the increasing levels of invasion. Therefore, only with community level studies that assess impacts in higher trophic levels and consider trophic relations among species (*i.e.* food webs), can the full impact of invasive species begin to be fully assessed.

Extinction simulation studies of food webs (Dunne *et al.* 2002; Memmott *et al.* 2004) assume that as long as one resource species is still available, consumer species will remain in the food web. For this to happen in real situations, the remaining resources need to be enough to sustain consumer populations (*i.e.* remain above the parametric threshold, Bascompte 2003). However, this study revealed that herbivore subsets suffered declines in species richness, even when the species richness of their resources (plants) was not significantly affected by the alien plant. Declines of diversity were also detected in parasitoid level. These results suggest that if reductions in resource abundances are strong enough, local extinction of insects could occur before all resource species are lost from the network. Therefore, extinction simulations such as the ones carried out by Dunne *et al.* (2002) and Memmott *et al.* (2004), could underestimate impacts of disruptions.

Q3: Can the impact of* G. shallon *on consumers be inferred from its effect on resources?

As detected in previous studies on impacts of fragmentation and habitat simplification (Zabel & Tscharntke 1998; Rand & Tscharntke 2007), generalist consumer subsets were less affected by disturbances (in this case, of *G. shallon* invasion) than specialist consumer subsets. Although it has been demonstrated that contrasting responses to habitat changes can occur between generalist groups (*e.g.* coccinelid and spider predators) and specialist groups (*e.g.* parasitic wasps and cecidomyiid midges) of natural enemies (Rand & Tscharntke 2007), this study demonstrates for the first time

differences in susceptibility in specialist and generalists consumers *within* the same group of natural enemies (*i.e.* parasitic wasps).

Generalist consumer subsets were less affected than their resources (Scenario 1), which suggests that they were able to adjust their diet and use their unaffected resources to maintain their abundance. As for specialist consumers, although both subsets (herbivore and parasitoid) were negatively affected by *G. shallon*, they also differed in their response to resource decline. While specialist parasitoids were affected to the same extent as their resources, with no changes in density relative to resources (Scenario 2), specialist herbivores were significantly more affected than their resources, declining in density relative to resources (Scenario 3). Therefore, declines in resources were only a good indicator of impacts in higher trophic levels for specialist parasitoid subsets, overestimating impacts on generalist consumer subsets and underestimating impacts on specialist herbivore subsets. It is possible that parasitoids are less susceptible to being affected to resource declines than herbivores. However, the divergence of results in the two specialist consumer communities (herbivores and parasitoids) may also be explained by the fact that while specialist herbivores had all their resources (native Ericaceae) affected by the invasion of *G. shallon*, specialist parasitoid species fed on both affected (specialist herbivores) and unaffected (generalist herbivores) members of herbivore families. Therefore, specialist parasitoids may use generalist herbivores as refugia, allowing them to maintain their densities. Research on plant-aphid-parasitoid communities in agro-ecosystems has shown a similar effect, with specialist herbivores being affected more than their resources (*i.e.* density decline relative to their resources), while specialist parasitoids decreased in proportion to their specialist host (Rand & Tscharntke 2007). Similar to my study, their focal specialist herbivore (the aphid, *Microlophium carnosum*) is monophagous and had all its resources (*Urtica dioica*) affected by habitat simplification, and their most abundant specialist parasitoid (*Aphidius matricariae*) is a broadly oligophagous species, being able to parasitize several aphid species (Zamani *et al.* 2006; Lumbierres *et al.* 2007) that are possibly undisturbed by habitat simplification. Moreover, my results reveal that, even when both specialist and generalist parasitoid subsets have alternative unaffected resources (*i.e.* generalist herbivores), specialist parasitoids do not maintain their abundances (Scenario 2), as generalist parasitoids do (Scenario 1). In conclusion, both diet breath and the

proportion of resources affected by the invasion are likely to have a key role in the regulation of the propagation of impacts through plant-herbivore-parasitoid food webs.

Extinction simulation studies (*e.g.* Dunne *et al.* 2002; Memmott *et al.* 2004) aim to predict the community level consequences of local species extinction (*i.e.* species loss from the network). The results of this work indicate that a reduction in abundance of a resource is enough to elicit a knock on effect on the abundance of certain specialist consumers. Thus, although the existing extinction simulation studies may provide accurate predictions for systems where generalist species are the most frequent (*e.g.* plant-pollination food webs), and hence more likely to adapt their diet, they may underestimate impacts on more specialized communities. Any predictions can, however, be improved if they consider consumer responses to declines on resource abundance. Based on the results presented here, I suggest two general rules to evaluate quantitatively the community level consequences of declines in a resource species (see schematic representation in Fig 3.4):

1) Evaluation of diet breadth: generalist species will adapt their diets (by changing frequency of use of their resources), maintaining their abundances (Scenario 1); while specialists will not adapt their diet and, thus, decline in abundance.
2) Quantification of the proportion of resources affected for each consumer, to enable the decline on specialists to be predicted: specialist species that have alternative resources will decline in abundance only in proportion to their resources (Scenario 2), while species that have all their resources affected will decline more in abundance than their resources (Scenario 3).

Therefore, species that have only part of their resources affected will only go extinct if all resources are lost (as previous extinction models predict), while species that have all their resources affected may go extinct before their resources are totally lost from the network. However, if all resource species are affected, all consumer species (generalist or specialist) are expected to suffer density declines (Scenario 3) and hence go extinct before their resources. Information on extinction thresholds of the species involved would allow a more precise identification of the proportion of resources that can be lost before a species goes locally extinct. More research is needed to test these general rules

in a wide variety of ecosystems, involving different guilds (*e.g.* pollinators, frugivorous birds).

Implications of the results for ecosystem conservation

The results of this work show that impacts of invasive plants can propagate up through the food web, leading to changes in community structure and loss of trophic diversity. Implications of the loss of trophic diversity can be profound as specialist natural enemies may have a stronger regulating role on resource abundance than generalist natural enemies (Hassell & May 1986; Closs 1999). Thus, generalization of invaded communities may disrupt top-down regulatory forces, which may lead to herbivore species outbreaks.

Previous studies suggest that higher trophic levels may provide a better proxy when estimating the health of an ecosystem than the lower levels (reviewed by Hudson *et al.* 2006). However, this study indicates that it is important to consider the specialization level of the species if they are to be used as bio-indicators, as generalist species might indicate that impacts are attenuated, while specialist species may indicate magnification of impacts.

Finally, as disturbance by invasive species is leading to a more generalized community, and as previous studies have shown that higher connectance on food webs increases its robustness to impacts (Dunne *et al.* 2002), disturbed communities might become more robust to future disruptions. This result may have important implications in habitat restoration, as it suggests that disturbed generalized communities might be resilient to efforts attempting to restore the original trophically diverse ecosystems.

Concluding remarks

This study highlights the fragility of highly specialized communities to disturbances, such as plant invasion, providing new insights into the regulatory mechanisms of food webs and revealing important changes in community structure.

These results may have implications for biocontrol services provided by natural enemies, for the selection of species to be used as bio-indicators, as well as for the restoration of habitats. Moreover, this study reveals that by integrating information on both diet breadth and on the proportion of affected resources, predictive studies that evaluate consequences of ecosystems disruptions can be improved. This information can be provided by quantitative food webs, such as the ones presented here.

Tables and Figures

Table 3.1 Impact of *G. shallon* on native communities of plants, herbivores and parasitoids. Values indicating the strength of *G. shallon* impact (β value) and adjusted R^2 (aR^2) values are presented whenever *G. shallon* abundance had a significant effect. *P* values presented were obtained from likelihood ratio F-tests comparing deviances with and without *G. shallon* abundance in the model with all eight plots as well as in model with only the seven plots below 50% invasion. ns: *P* value > 0.05.

		all plots				plots below 50% invasion	
Community	**Response variable**	**β**	**$F_{1,6}$**	**P**	**aR^2**	**$F_{1,5}$**	**P**
Plants	Abundance	- 0.0133	26.9	0.0021	0.79	6.2	ns
	Species richness	-	0.5	ns	-	0.5	ns
	Evenness	0.0067	129.9	<0.0001	0.95	46.2	0.0011
Herbivores	Abundance	- 0.0401	58.1	0.0003	0.89	28.2	0.0032
	Species Richness	- 0.0083	11.5	0.0148	0.60	0.2	ns
	Proportion of generalist species	- 0.0067	21.6	0.0035	0.75	21.1	0.0059
	Evenness	0.0072	17.8	0.0056	0.71	14.8	0.0120
Parasitoids	Abundance	- 0.0404	28.4	0.0018	0.80	10.3	0.0236
	Species richness	- 0.0118	6.2	0.0474	0.43	0.6	ns
	Proportion of generalist species	- 0.0025	7.8	0.0318	0.49	11.6	0.0192
	Evenness	0.0037	21.0	0.0038	0.74	8.3	0.0348

Table 3.2 Impact of *G. shallon* on the abundance of the consumer subsets and their resources. Values indicating the strength of *G. shallon* impact (β value) and adjusted R^2 (aR^2) values are presented whenever *G. shallon* abundance had a significant effect. *P* values presented were obtained from likelihood ratio F-tests comparing deviances with and without *G. shallon* abundance in the model with all eight plots as well as in model with only the seven plots below 50% invasion. G Herb: generalist herbivores; S Herb: specialist herbivores; G Par: generalist parasitoids; S Par Chry: Chrysomelidae specialist parasitoids; S Par Noc: Noctuidae specialist parasitoids; S Par Geo: Geometridae specialist parasitoids; N plants: native plants; Eric: Ericaceae; Lep: Lepidoptera; Chry: Chrysomelidae; Noc: Noctuidae; Geo: Geometridae; ns: *P* value > 0.05.

	all plots				plots below 50% invasion	
Consumer subset	**β**	**$F_{1,6}$**	**P**	**aR^2**	**$F_{1,5}$**	**P**
G Herb	-	0.2	ns	-	2.7	ns
S Herb	-0.04	57.3	0.0002	0.89	27.8	0.0033
G Par	-	5.1	ns	-	0.2	ns
S Par Chry	-0.04	26.7	0.0021	0.79	10.3	0.0236
S Par Noc	-0.02	9.2	0.0231	0.54	13.0	0.0155
S Par Geo	-0.02	8.3	0.0279	0.51	24.3	0.0044

	all plots				plots below 50% invasion	
Resource	**β**	**$F_{1,6}$**	**P**	**aR^2**	**$F_{1,5}$**	**P**
Nplants	-0.01	26.9	0.0021	0.79	6.2	ns
Eric	-0.02	43.4	0.0006	0.89	19.7	0.0068
Lep	-0.03	42.7	0.0006	0.86	12.7	0.0163
Chry	-0.04	39.1	0.0008	0.84	25.7	0.0039
Noc	-0.02	53.3	0.0003	0.88	11.1	0.0209
Geo	-0.03	11.1	0.0158	0.59	0.0	ns

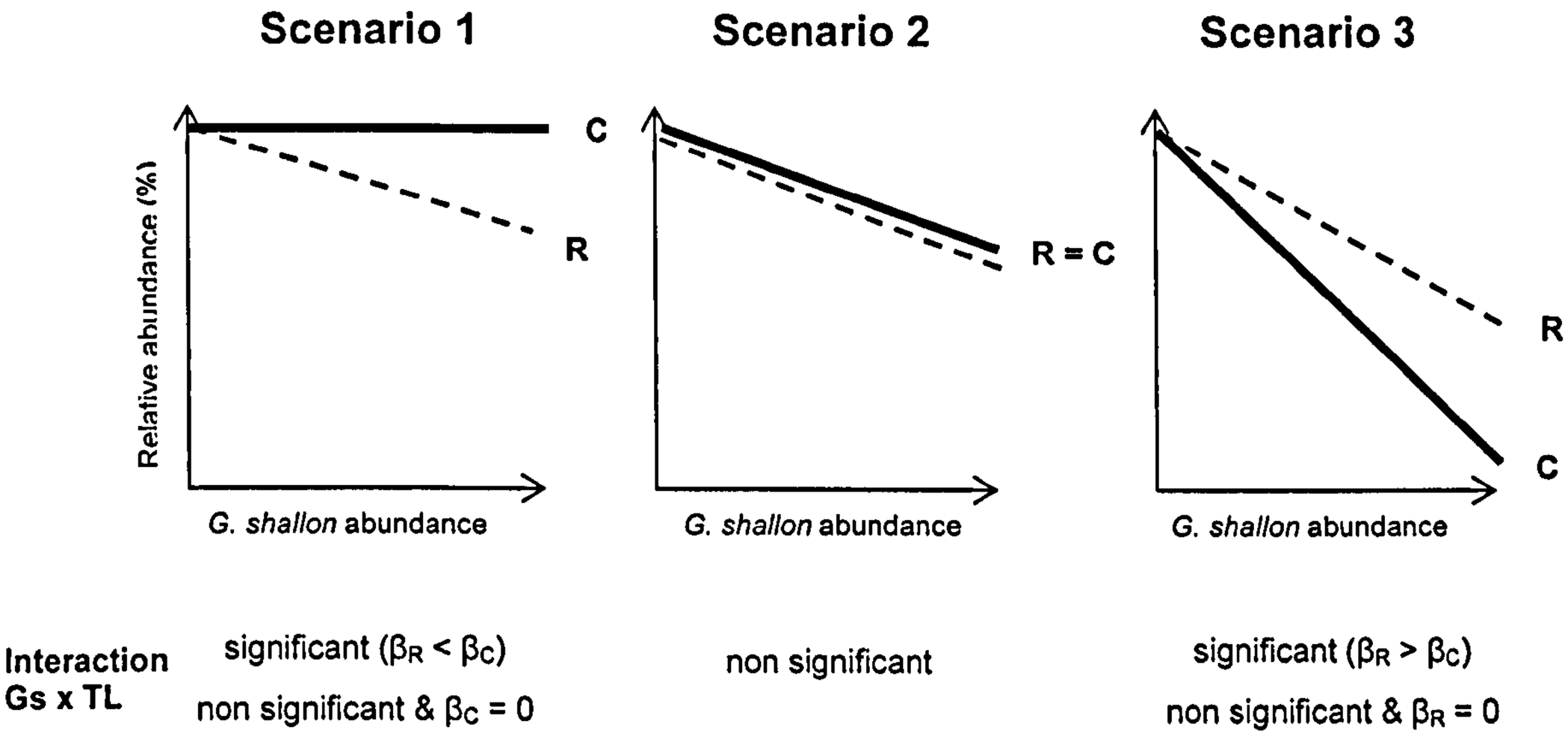

Figure 3.1 The three scenarios that describe how consumers could respond to resource decline. Lines in graphs represent the effect of *G. shallon* (**Gs**) in the two levels (Consumer, **C** and resource, **R**) of the factor Trophic level (**TL**). Statistical results which would validate each scenario are described below the graphs, where the significance or non significance of an F-test on deviances with and without the interaction term is presented together with expected values for the slope of each level (β_R – slope of resources; β_C- slope of consumers).

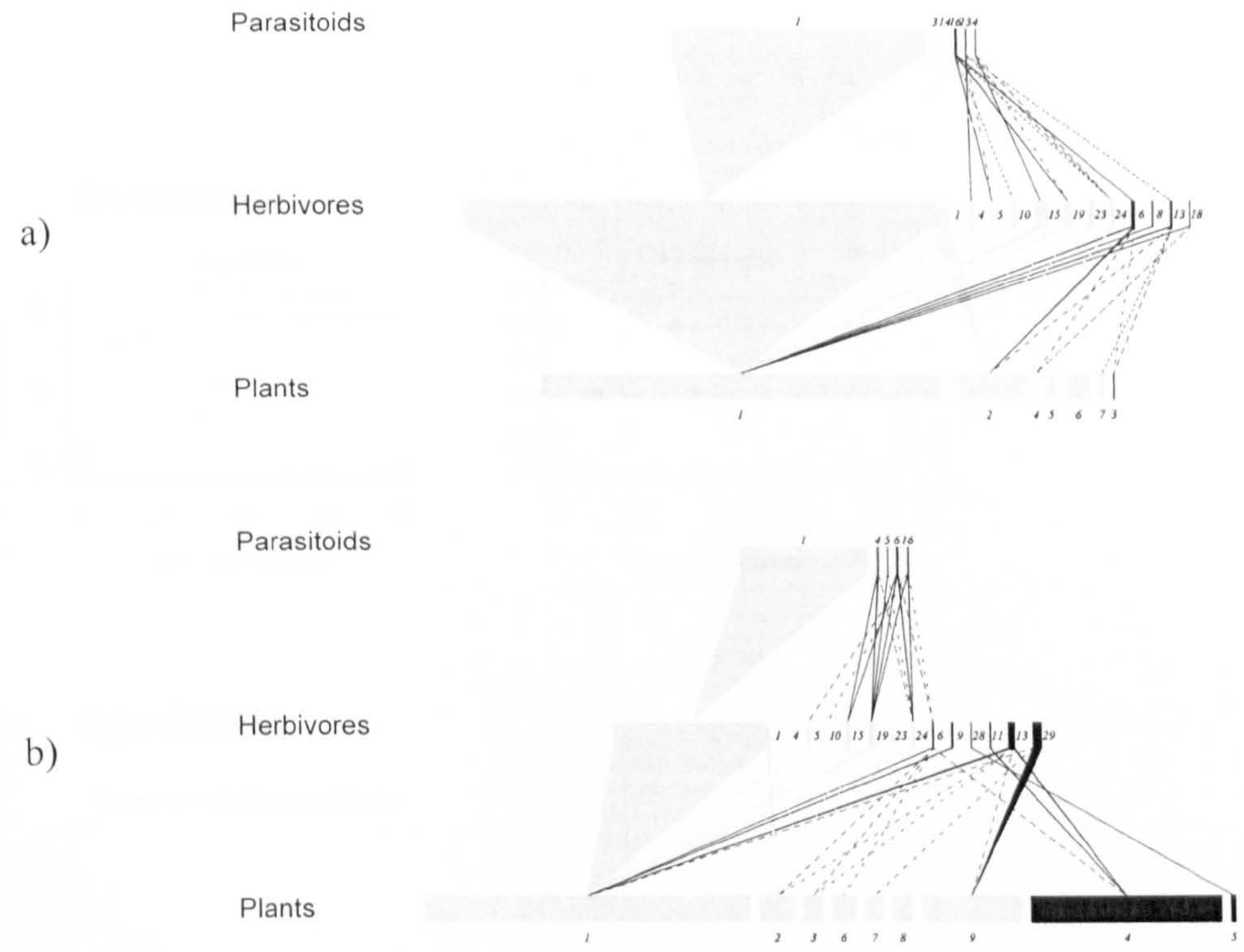

Figure 3.2 Plant-herbivore-parasitoid food web for three plots representative of the pattern found along *G. shallon* invasion gradient for all eight plots, a) 0.2% *G. shallon*; b) 30.0% *G. s*; c) 79.2% *G. s*. Each species is represented by a rectangle. The widths of the rectangles are proportional to their abundance at the field site and the size of the triangles connecting them represents the frequency of interactions in the study area. Dotted lines represent interactions reported in the literature, but which were not detected during the sampling surveys. Invasive plants (*G. shallon* and *R. ponticum*) are shown in black as well as generalist consumers (herbivores and parasitoids). Plant, herbivore and parasitoid names are provided in Tables S3.2-4.

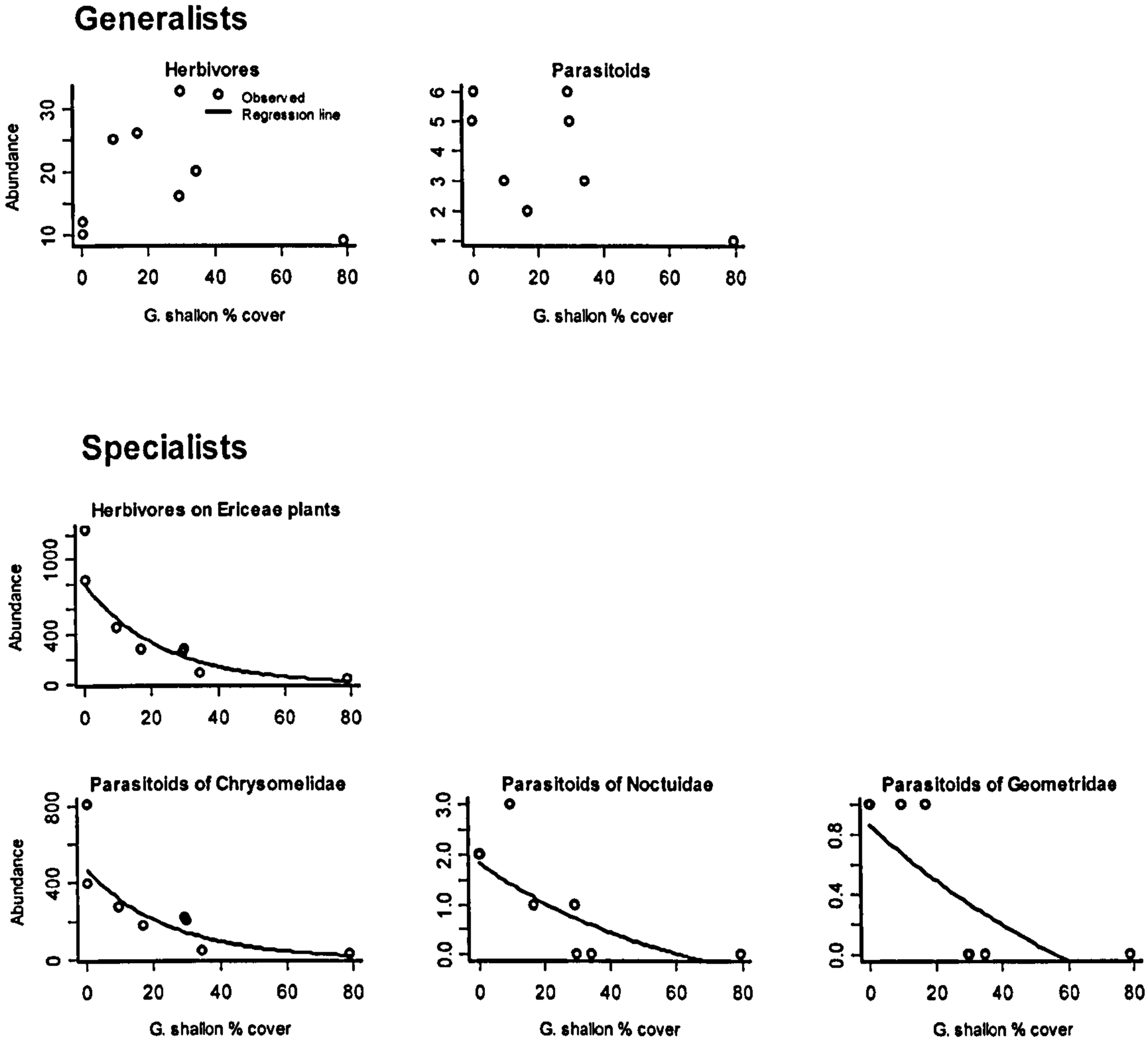

Figure 3.3 Effect of *G. shallon* on the abundance (number of individuals) of specialist and generalist consumer subsets. Regression lines (untransformed from log transformations) are presented whenever the effect of *G. shallon* on the response variable is significant. Model details are provided in Table 3.2.

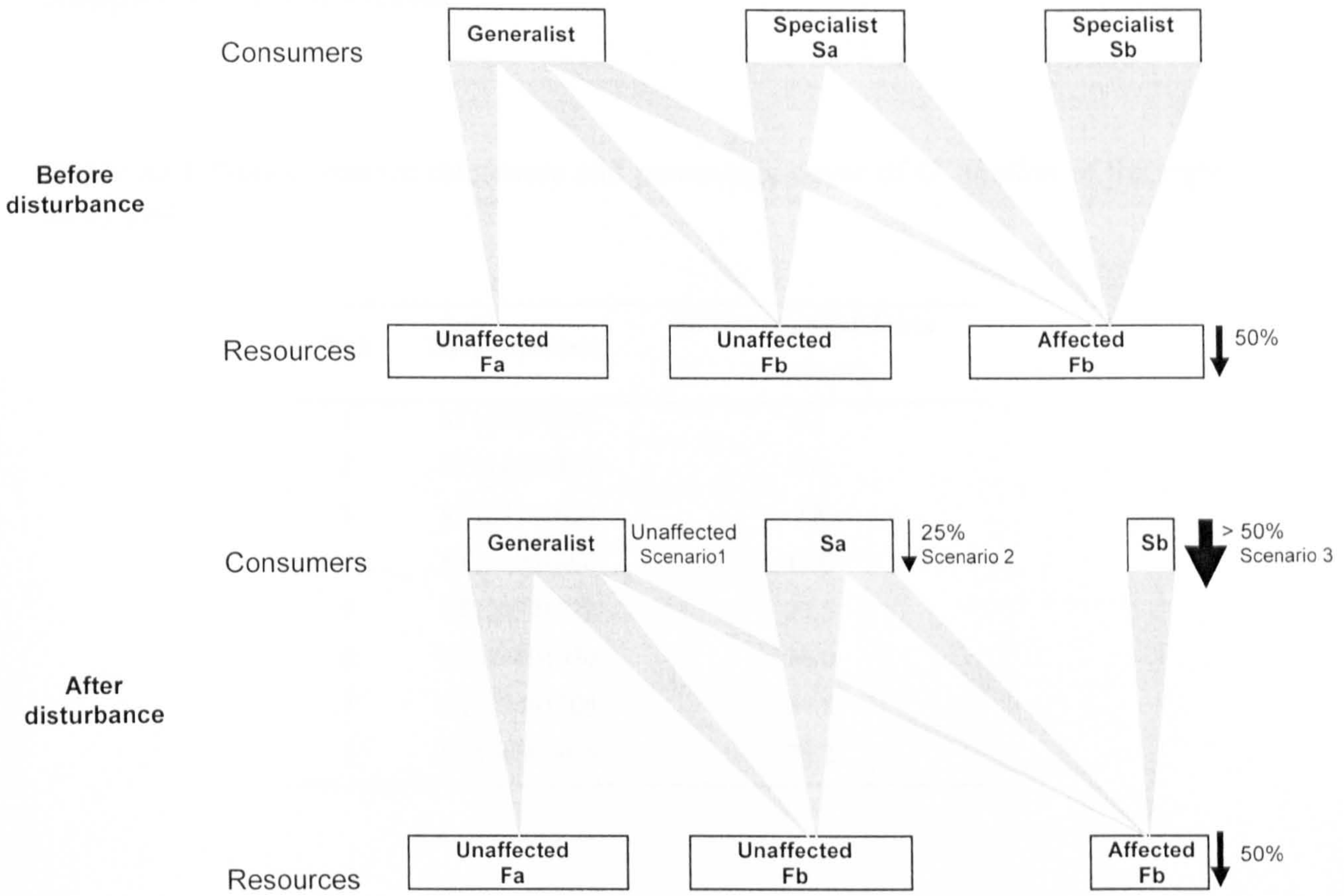

Figure 3.4 Schematic representation of community level changes, based on the two general rules suggested in this study. Resource-consumer food web before and after disturbance. Each species is represented by a rectangle. The widths of the rectangles are proportional to their abundance at the field site and the size of the triangles connecting them represents the frequency of interactions in the study area. Arrows represent declines in abundance. Fa – resource family Fa; Fb – resource family Fb.

Supplementary material

Table S3.1 Plots Ordnance references and percentage cover of *G. shallon* of the eight study plots.

Plot	Grid reference	*G. shallon* abundance (% cover)
1	ST125001300	0.2
2	ST124001475	0.4
3	ST124101600	9.8
4	ST123001400	17.0
5	ST121501520	29.5
6	ST126001050	30.0
7	ST123401200	34.8
8	ST126801405	79.2

Table S3.2 Plant species of the food web. The code of the species corresponds to the code in the food web diagram (Fig 3.2). * - introduced species.

Code	Plant	Subfamily	Overall abundance (% cover)
1	*Calluna vulgaris* (L.) Hull	Ericaceae	69.4
2	*Erica cinerea* L.	Ericaceae	11.4
3	*Erica tetralix* L.	Ericaceae	1.2
4	*Ulex europaeus* L.	Fabaceae	0.1
5	*Ulex minor* Roth	Fabaceae	5.4
6	*Cytisus scoparius* (L.) Link	Fabaceae	0.1
7	*Betula* sp.	Rosaceae	1.7
8	*Rubus fruticosus* L.	Rosaceae	0.01
9	*Pinus* sp.	Pinaceae	3.4
10	*Pteridium aquilinum* (L.) Kuhn	Hypolepidaceae	10.2
11	*Gaultheria shallon* Pursh *	Ericaceae	17.5
12	*Rhododendron ponticum* L. *	Ericaceae	0.1

Table S3.3 Herbivore species of the food web. The code of the species corresponds to the code in the food web diagram (Fig 3.2). S - Specialist species (*i.e.* feeding only on one tribe of plant species); Col – Coleoptera, Lep – Lepidoptera, G – generalist species (*i.e.* feeding on more than one family of plant species), Eric S – specialist on Ericaceae specialist, Bet S – specialist on Betulaceae; und –diet breadth undetermined.

Code	Herbivore	Family	Diet breadth	Plant resources in the study area
1	*Lochmaea suturalis* Thomson	Col: Chrysomelidae	Eric S	*Calluna vulgaris*
				Erica cinerae
				Erica tetralix
2	*Arctia caja* L.	Lep: Arctidae	G	*Calluna vulgaris*
				Pteridium aquilinum
				Rubus fruticosus
3	*Phragmatobia fuliginosa* L.	Lep: Arctiidae	G	*Calluna vulgaris*
4	*Aristotelia ericinella* Zeller	Lep: Gelechiidae	Eric S	*Calluna vulgaris*
				Erica cinerae
5	*Neofaculta ericetella* Geyer	Lep: Gelechiidae	Eric S	*Calluna vulgaris*
				Erica cinerae
				Erica tetralix
6	*Ematurga atomaria* L.	Lep: Geometridae	G	*Betula* sp.
				Calluna vulgaris
				Erica cinerae
				Erica tetralix
				Gaultheria shallon
7	*Epirrita christyi* Allen	Lep: Geometridae	G	*Calluna vulgaris*
8	*Eupithecia absinthiata f. goossensiata* Clerck	Lep: Geometridae	Eric S	*Calluna vulgaris*
9	*Eupithecia centaureata* cf. Denis & Schiff.	Lep: Geometridae	G	*Calluna vulgaris*
10	*Eupithecia nanata* Hübner	Lep: Geometridae	Eric S	*Calluna vulgaris*
				Erica cinerae
11	Geometridae sp1	Lep: Geometridae	und	*Gaultheria shallon*
13	*Gymnoscelis rufifasciata* Haworth	Lep: Geometridae	G	*Calluna vulgaris*
				Cytisus scoparius
				Erica cinerae
				Gaultheria shallon
				Pteridium aquilinum
				Ulex europaeus
14	*Idaea dimidiata* Hufnagel	Lep: Geometridae	G	*Gaultheria shallon*
15	*Pachycnemia hippocastanaria* Hübner	Lep: Geometridae	Eric S	*Calluna vulgaris*
				Erica cinerae
				Erica tetralix
16	*Lasiocampa quercus* L.	Lep: Lasiocampidae	G	*Calluna vulgaris*
				Cytisus scoparius
				Rubus fruticosus
17	*Macrothylacia rubi* L.	Lep: Lasiocampidae	G	*Calluna vulgaris*
				Gaultheria shallon
18	*Dicallomera fascelina* L.	Lep: Lymantriidae	G	*Betula* sp.
				Calluna vulgaris
				Cytisus scoparius

	Dicallomera fascelina L. (cont)			*Rubus fruticosus*
19	*Anarta myrtilli* L.	Lep: Noctuidae	Eric S	*Calluna vulgaris*
				Erica cinerae
20	*Autographa gamma* L.	Lep: Noctuidae	G	*Gaultheria shallon*
21	*Ceramica pisi* L.	Lep: Noctuidae	G	*Betula* sp.
				Calluna vulgaris
				Cytisus scoparius
22	*Lacanobia w-latinum* Hufnagel	Lep: Noctuidae	G	*Cytisus scoparius*
				Gaultheria shallon
23	*Lycophotia porphyrea* Denis & Schiff.	Lep: Noctuidae	Eric S	*Calluna vulgaris*
				Erica cinerae
				Erica tetralix
24	*Xesthia agathina* Dup. cf.	Lep: Noctuidae	Eric S	*Calluna vulgaris*
				Erica cinerae
25	*Papestra biren* Goeze	Lep: Noctuidae	G	*Calluna vulgaris*
26	*Pheosia gnoma* Fabricius	Lep: Notodontidae	Bet S	*Betula* sp.
27	*Argyrotaenia ljungiana* Thunberg	Lep: Tortricidae	G	*Calluna vulgaris*
28	Unidentified lepidoptera	Lepidoptera	und	*Rhododendron ponticum*
29	Symphita sp	Hymenoptera	G	*Calluna vulgaris*
				Pteridium aquilinum
				Ulex minor

Table S3.4 Parasitoid species of the food web. The code of the species corresponds to the code in the food web diagram (Fig 3.2). S - Specialist species (*i.e.* feeding only on one tribe of plant species); Hym – Hymenoptera, Dip – Diptera, G – generalist species (*i.e.* feeding on more than one family of host species); Chry S – specialist on Chrysomelidae; Geo S - specialist on Geometridae; Noct S – specialist on Noctuidae; und –diet breadth undetermined.

Code	Parasitoid	Family	Diet breadth	Hosts present in study area
1	*Asecodes mento* Walker	Hym: Eulophidae	Chry S	*Lochmaea suturalis*
2	*Aleiodes alternator* Nees	Hym: Braconidae	G	*Arctia caja*
				Phragonatobia fuliginosa
				Macrothylacia rubi
				Lasiocampa quercus
3	*Cotesia errator* Nixon	Hym: Braconidae	Geo S	*Eupithecia nanata*
4	*Meteorus colon* Haliday	Hym: Braconidae	G	*Anarta myrtillis*
				Xesthia agathina cf.
				Pachycnemia hippocastanaria
5	*Meteorus pulchricornis* Wesmael	Hym: Braconidae	G	*Anarta myrtillis*
				Xesthia agathina cf.
6	*Meteorus versicolor* Wesmael	Hym: Braconidae	G	*Anarta myrtillis*
				Xesthia agathina cf.
				Pachycnemia hippocastanaria
				Ematurga atomaria
7	*Barylypa propagnator*	Hym: Ichneumonidae	Noct S	*Anarta myrtillis*
8	*Campoletis latrator* Gravenhorst	Hym: Ichneumonidae	und	*Xesthia agathina* cf.
9	*Campoletis rapax* Bridgman	Hym: Ichneumonidae	und	*Anarta myrtillis*
10	*Campoletis* sp1	Hym: Ichneumonidae	und	*Gymnoscelis rufifasciata*
11	*Campoplex* sp1	Hym: Ichneumonidae	und	*Neofaculta ericetella*
12	*Chorinaeus* sp1	Hym: Ichneumonidae	und	*Neofaculta ericetella*
13	*Diadegma armillata* Gravenhorst	Hym: Ichneumonidae	G	*Aristotelia ericinella*
				Neofaculta ericetella
				Xesthia agathina cf.
				Gymnoscelis rufifasciata
14	*Hyposoter didymator* Thunberg	Hym: Ichneumonidae	Noct S	*Anarta myrtillis*
				Lycophotia porphyrea
				Xesthia agathina cf.
				Papestra biren
15	*Meloboris collector* Thunberg	Hym: Ichneumonidae	Noct S	*Xesthia agathina* cf.
16	*Campylocheta inepta* Meigen,	Dip: Tachinidae	G	*Anarta myrtillis*
				Eupithecia nanata
				Neofaculta ericetella
				Xesthia agathina cf.
				Ematurga atomaria
17	*Athrycia impressa* van der Wulp	Dip: Tachinidae	G	*Anarta myrtillis*
				Ematurga atomaria

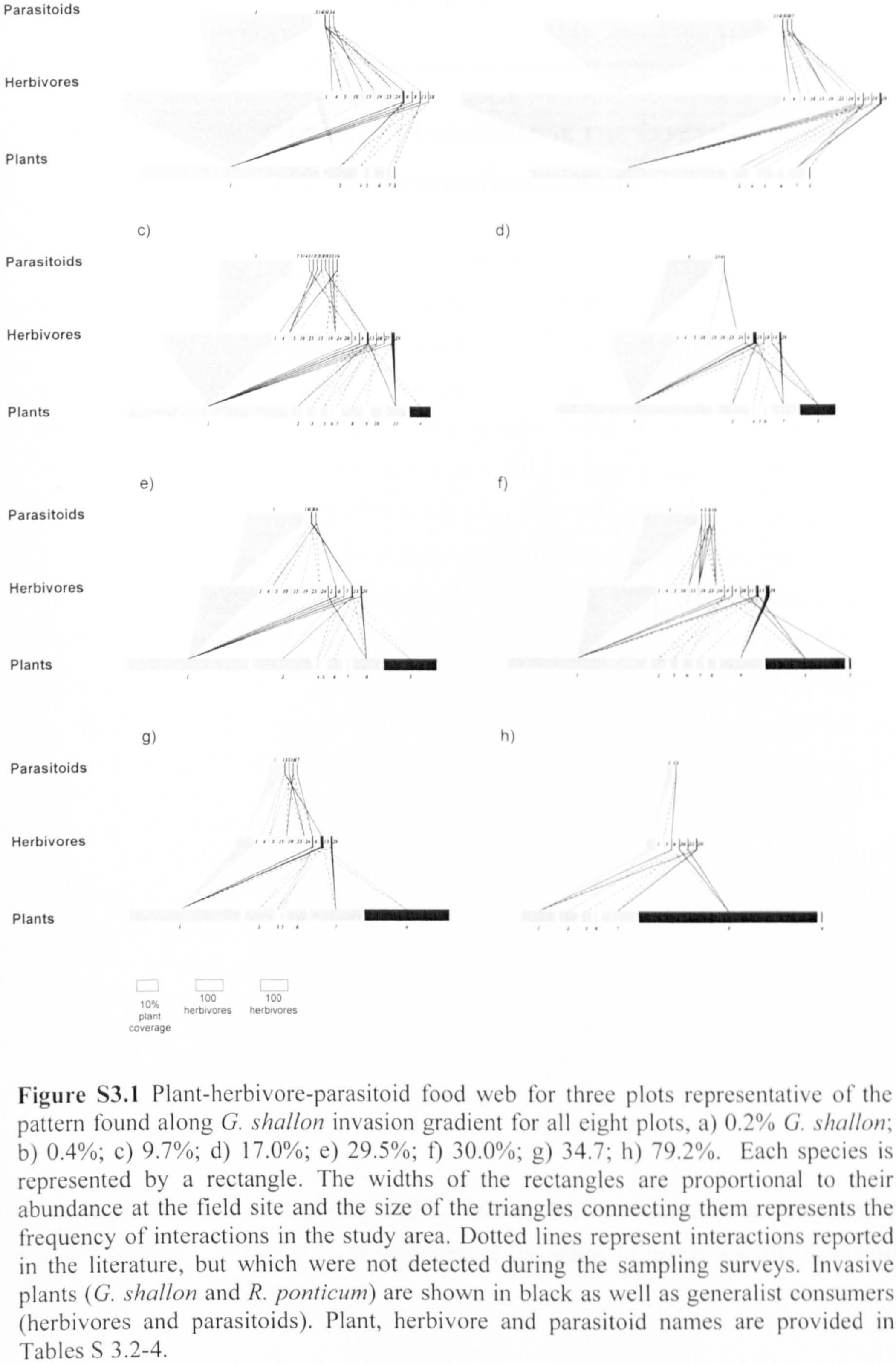

Figure S3.1 Plant-herbivore-parasitoid food web for three plots representative of the pattern found along *G. shallon* invasion gradient for all eight plots, a) 0.2% *G. shallon*; b) 0.4%; c) 9.7%; d) 17.0%; e) 29.5%; f) 30.0%; g) 34.7; h) 79.2%. Each species is represented by a rectangle. The widths of the rectangles are proportional to their abundance at the field site and the size of the triangles connecting them represents the frequency of interactions in the study area. Dotted lines represent interactions reported in the literature, but which were not detected during the sampling surveys. Invasive plants (*G. shallon* and *R. ponticum*) are shown in black as well as generalist consumers (herbivores and parasitoids). Plant, herbivore and parasitoid names are provided in Tables S 3.2-4.

Chapter 4

APPARENT COMPETITION CAN COMPROMISE THE SAFETY OF HIGHLY SPECIFIC BIOCONTROL AGENTS

In: **Carvalheiro, L. G.**, Buckley, Y.M., Ventim, R., Fowler, S. V. & Memmott, J. (2008). Apparent competition can compromise the safety of highly specific biocontrol agents. *Ecology Letters* (in press).

Chapter 4

APPARENT COMPETITION CAN COMPROMISE THE SAFETY OF HIGHLY SPECIFIC BIOCONTROL AGENTS

Summary: Despite current concern about the safety of biological control of weeds, assessing the indirect impacts of introduced agents is not common practice. Using 17 replicate food webs I demonstrate, for the first time, that the use of a highly host-plant specific weed biocontrol agent, recently introduced into Australia, is associated with local biodiversity losses. The agent shares natural enemies (predators and parasitoids) with seed herbivore species from native plants, so apparent competition is the most likely cause for these losses. Both species richness and abundance in insect communities were negatively correlated with the abundance of the biocontrol agent. Local losses of up to 11 species (dipteran seed herbivores and parasitoids) took place as the biocontrol agent abundance increased. Ineffective biocontrol agents that remain highly abundant in the community are most likely to have persistent, indirect negative effects. These findings suggest that more investment is required in pre-release studies on the effectiveness of biocontrol agents, as well as in post-release studies assessing indirect impacts, to avoid or minimize the release of potentially damaging species.

Key words: biological control, apparent competition, native communities, herbivory, natural enemies, Bitou, *Mesoclanis polana*.

Introduction

Biological control has been widely used in the management of plant invasions, and the safety of biocontrol (*i.e.* likelihood of causing ecological damage) has received considerable attention (*e.g.* Simberloff & Stiling 1996a; 1996b; Lynch *et al.* 2002; Dudley & Kazmer 2005; Hufbauer & Roderick 2005). It has been argued that, with current practices, negative non-target impacts of biological control are occasional and predictable (Sheppard *et al.* 2006). However, the impacts of introduced agents on native species via apparent competition (*i.e.* competition due to shared natural enemies (Holt & Lawton 1994)) have been little studied in a biocontrol context (Willis & Memmott 2005). A successfully established biocontrol agent can become an abundant food source for generalist natural enemies present in the target ecosystem. Natural enemies (*e.g.* parasitoids or pathogens) which include the biocontrol agent in their diet may themselves become more abundant (*e.g.* Willis & Memmott 2005; Pearson & Callaway 2006). Apparent competition between the biocontrol agent and native species may thus occur (Tompkins *et al.* 2000; Muller & Godfray 1997; Morris *et al.* 2001; Redman & Scriber 2000) and may in turn have important negative effects on local fauna and flora. For example, Pearson & Callaway (2006) revealed that by providing food for populations of deer mice, the primary reservoir for hantavirus, two host specific biocontrol agents may increase risk of the virus infection in other virus hosts, including humans. Such negative effects could prove particularly problematic for conservation if rare endemic native species are affected (van Veen *et al.* 2006).

Chrysanthemoides monilifera ssp. *rotundata* (L.) T. Nord (henceforth Bitou) is a South African member of the Asteraceae, which is highly invasive and a serious threat to native biodiversity on the eastern coast of Australia (Weiss & Noble 1984a, 1984b). In 1996, a highly specific seed herbivore of Bitou, *Mesoclanis polana* (Munro) (Diptera: Tephritidae), was released as a biocontrol agent (Edwards & Brown 1997). Although it spread very successfully throughout the entire range of Bitou (Edwards *et al.*1999), it is ineffective as a biocontrol agent, because levels of seed predation are too low to impact upon the population dynamics of Bitou (Noble & Weiss 1989; Adair & Bruzzese 2000). In 1998, parasitism was detected at relatively high rates in *M. polana* in New South Wales (NSW) (Willis & Memmott 2005), demonstrating the potential for apparent competition between the biocontrol agent and native herbivore hosts of their

shared natural enemies. Here I use a food web approach to quantify the indirect impacts of a host-specific biocontrol agent on non-target insect and plant communities. I aim, firstly, to identify, and quantify the abundance of, any natural enemies that *M. polana* shares with other seed herbivores. If abundant shared natural enemies exist, apparent competition and therefore negative effects on biodiversity are likely. Secondly, I aim to determine whether the abundance of *M. polana* is correlated with the abundance and species richness of seed herbivores, parasitoids and plants from the native community. If negative correlations exist, negative effects of the use biocontrol agents on biodiversity are demonstrated for the first time.

Methods

The food web

Seventeen 1600m^2 plots were selected according to accessibility along an 84 km stretch of NSW coast to sample a gradient of Bitou (and therefore *M. polana*) abundance in the four different habitats where Bitou is present (headlands, heathlands, dunes, and coastal forest, see grid references in Table S4.1 of Supplementary Material). Plots were separated by 500m to 84 km. The maximum level of Bitou allowed per plot was 50% of the total plant coverage area, as the aim of this work was to study the native insect communities that depend on native plants. Bitou is actively controlled throughout its range by using herbicide, so the Bitou abundance gradient is associated with variation in the intensity of the herbicide treatment used to control the plant, a high abundance indicating a low level of chemical control. To minimize the influence of the herbicides used to control Bitou, all fieldwork was carried out in the three months before the annual herbicide application.

Plots were sampled for seeds, seed herbivores and natural enemies by using standard food web sampling and rearing methods (Willis & Memmott 2005). A total of three surveys were carried out on each plot between 1st April and 30th June 2006. This period is the Australasian autumn and covers one of the fruiting peaks of Bitou (Gosper 2004) and at least part of the fruiting season of the majority of the plant species present

in the plots (Carvalheiro, personal observation). In each survey, three non-overlapping parallel transects, each 3m wide and 20m long, were placed at random within the plot. The abundance of each plant species present in each transect was recorded as a percentage of the total plant coverage area. All seed units (a unit being needed for a large seed herbivore larva to develop, *e.g.* in Asteraceae, each inflorescence produces one seed unit) were collected and kept in individual 1mm microperforated polypropylene bags. A piece of absorbent paper was placed in each bag, to maintain a dry environment until the seed herbivores or their natural enemies emerged. For plant species fruiting at very high densities, a sub-sample of seed units was collected and the proportion of all seeds of that species included in the sub-sample was recorded, so that data could be corrected for statistical analysis. Additional sampling was carried out in the plots, and in two other locations (Angourie Point, and Border Ranges National Park (BRNP)) to increase the sample size of seeds from plant species rarely encountered during the surveys. If a new interaction was found from the additional sampling in one of the seventeen plots, it was included in the dataset as a rare event (frequency of interaction = 0. 1). New interactions from Angourie Point and BRNP were included in the general food web to provide a more detailed survey of the links between species, but were not included in the data analysis.

Seed units collected from different plant individuals were kept separately and maintained in laboratory conditions for ten weeks. They were then checked two to three times per week during the first three weeks after collection, then weekly thereafter, for emergent insects. Reared insects were placed in 70% ethanol, except Lepidoptera which were frozen and later pinned. Ten weeks after collection I considered that the majority of insects had emerged. When a parasitoid was reared from a plant species from which no other insect had been reared, an interaction between the host plant and an unidentified seed herbivore was included in the dataset as a rare event (frequency of interaction = 0. 1).

All insects were identified by professional taxonomists to species or assigned to morphospecies, with the exception of some Lepidoptera, in which morphotyping was carried out by the authors. For natural enemy host identification, if only one seed herbivore species was present in the seed, interactions between species were identified directly from the rearing data. If more than one seed herbivore species was reared from a plant species from which a given parasitoid had been reared, information from the

literature on the host range of the parasitoid species was, in most cases, sufficient to identify the host. For example, if the parasitoid was known to feed only on Diptera, and dipteran and lepidopteran seed herbivores were found in the plant species, the parasitoid was assumed not to have used a lepidopteran as a host. In addition, to confirm these assumptions for the most common natural enemies reared from Bitou, Generalised Linear Models (GLMs) were used to assess the abundance of which of the available hosts (*M. polana* and Lepidoptera reared from Bitou) most influenced the abundance of those natural enemies. Only plots where Bitou seeds were present were included in this analysis. The most common natural enemies were defined those as present in at least five plots. Counts of abundances were log transformed and analysed with Gaussian errors and *F* tests on changes in deviance. Models were also tested for quadratic effects. For parasitoid species for which the host could not be clearly identified, all the seed herbivores found in the plant species from which the parasitoid species was reared were grouped together. In these cases, I can say without doubt that one or more of the seed herbivores of the plant was the parasitoid host. The rearing data from all 17 plots pooled were used to construct a general plant-herbivore-natural enemy food web, using software written in Mathematica™, and to describe how *M. polana* abundance is correlated with the abundance and species richness of the native communities, as described below.

Impact of M. polana *on native biodiversity*

GLMs were also used to test the effect of habitat and *M. polana* and Bitou abundance on both the abundance and species richness of native communities (consisting of plants, seed herbivores and parasitoids). Since taxa from the same Order are more likely to interact indirectly than those from different Orders (Lewis *et al.* 2002), the dipteran seed herbivores were also analysed separately. In this case, as no Diptera were reared from heathlands, this habitat was not included in the analysis. Counts of abundances and species were analysed by using a Poisson error distribution, log link function, and χ^2 tests of changes in deviance, or log transformed and analysed with Gaussian errors and *F* tests on changes in deviance if errors could be normalised. As native plant abundance was measured as a percentage of the coverage area, arcsin transformation was used to

normalise residuals. Models were also tested for quadratic effects. Overdispersion was accounted for by using quasi-Poisson error distributions where appropriate. By testing for significant differences in abundance and species richness of native plants and native insects between plots with and without the herbicide treatment, I confirmed that herbicide treatment had no effect the native ecosystem. Therefore, it was not included in the model procedure. Three species of fungus-feeding Coleoptera (two Lathridiidae species and *Sericoderus* sp. (Corylophidae)) and a facultative hyperparasitoid species (*Ormyrus* sp.) were reared in very low numbers, but were not included in the analysis, the former because they were not part of the seed feeding network, the latter because it was impossible to be sure which trophic level it was from. Data on invasive plant species and their associated insects were not included in the analyses, as the aim of the study was to determine the effect of *M. polana* on the native food web.

As *M. polana* abundance was always low in dunes (where low herbivory rates of *M. polana* were detected), headlands and heathlands (due to low abundances of Bitou), these three habitats were grouped together for the analysis. There were no significant differences between models in which habitats were grouped and models in which all habitats were considered separately. Data from the nine transects sampled throughout the season (3 transects x 3 survey visits) were pooled (rearing data) or averaged (plant coverage area) for each plot, as appropriate.

Backwards-forwards stepwise selection was used to test all possible combinations of predictor variables (Quinn & Keough 2002). Interactions between variables never contributed significantly to the fit of the model and therefore models including interactions are not presented. For each response variable, the most parsimonious model was selected as that with the lowest Bayesian Information Criterion, BIC (Burnham & Anderson 2002). BIC was used as the selection criterion instead of the Akaike Information Criterion, AIC, as BIC is a more conservative measure in which models with greater numbers of predictors are penalized (Rawlings *et al.* 1998). However, AIC values were also calculated, and ranks of models by AIC mostly agree with the BIC ranks.

As *M. polana* is a highly specific seed herbivore of Bitou, its abundance is always correlated with that of Bitou. If only one of these variables has a significant effect on native communities, but colinearity between the two is very high, each will be significant in models including either variable alone. In models including the abundance

of both the weed and the biocontrol agent, the effect of each variable may be non-significant. However, if colinearity is not complete and the effect of one explanatory variable (*e.g. M. polana* abundance) is strong enough, a significant effect of that variable will be detected over and above the effect of the other variable. Therefore, I always evaluate the effect of *M. polana* in models including Bitou abundance. In these models, significant *P*-values for *M. polana* represent the effect of *M. polana* on native communities over and above the effect of Bitou abundance. All statistical analyses were performed with the software R (R Development Core Team 2007).

Results

The food web

A total of 37,477 seed units, of which 27% were from Bitou, were collected from 60 plant species. Seventy-three species and morphospecies of seed herbivores, of which 15 were Diptera, were reared from the 60 plant species. Nine species were reared from Bitou seeds (*M. polana* along with eight Lepidoptera morphospecies). Two types of natural enemies were reared: a dipteran predator (*Lestodiplosis* sp., Cecidomyiidae) and hymenopteran parasitoids (42 species). Fifteen of the 42 species of parasitoids were reared from the herbivores on Bitou, and seven of these 15 species attack Tephritidae (Willis & Memmott 2005; Edwards 1998; NHM 2007b; Ovruski *et al.* 2004) or are typically Diptera parasitoids (*e.g. Leptopilina* sp., van Alphen *et al.* 1991). Given that *M. polana* made up 98% of herbivore rearings from Bitou and was the only dipteran herbivore reared from Bitou, it is highly likely that *M. polana* was the host for these eight parasitoid species, if not more. Moreover, in contrast to the Lepidoptera reared from Bitou, *M. polana* abundance had a significant positive effect on the abundance of each of the four most abundant species of natural enemies (Table 4.1). Using a conservative approach, all other parasitoid species (*i.e.* eight of the 15) reared from Bitou fruits were not counted as *M. polana* parasitoids, as I could not be sure whether they were reared from the Lepidopteran seed herbivores, from *M. polana* or from a rare seed herbivore not found in the 10,194 seed units. Overall, 12% of individuals of *M. polana* had been attacked by natural enemies. The maximum attack rate per plot was

24%. This level of parasitism led to a six-fold increase in the abundance of natural enemies in the community, from low to high abundances of *M. polana*. Where *M. polana* was abundant, the community became dominated by species able to use the agent as a host (Fig 4.1).

Both dipteran and hymenopteran natural enemies were shared by *M. polana* and seed herbivores from native plants (Fig 4.1). *Lestodiplosis* sp. was the third most abundant natural enemy in the plots and was reared from 11 plant species; 54% of the rearings came from Bitou. Although, no links with native plants were found for the four most abundant parasitoid species (*Eupelmus* (*Eupelmus*) sp., *Megastigmus* sp., *Pteromalus* sp. and *Torymoides kiesenwetteri* (reared from 49%, 30%, 13%, and 7% of the parasitized *M. polana* individuals, respectively), three less abundant hymenopteran parasitoids of *M. polana* were shared with seed herbivores reared from native plants.

Impact of M. polana *on native biodiversity*

Native seed herbivore community

The best models describing abundance and species richness of seed herbivores reared from native plants included the effect of habitat (coastal forest had significantly higher values for abundance and species richness than the three grouped habitats) and the effect of *M. polana* abundance, which had a significant negative effect (Table 4.2). However, in a suboptimal model in which both Bitou and *M. polana* abundance were included, no significant effect was detected for either of them. This suggests that while *M. polana* has more explanatory power than Bitou, the effect of *M. polana* on native seed herbivores is not strong enough to be significant, due to colinearity with Bitou. When only dipteran seed herbivores were considered, the best models obtained for abundance and species richness included significant effects of both *M. polana* abundance and habitat (Table 4.2). However, in this case the effect of the biocontrol agent was significant over and above the effect of Bitou (*i.e.* an F-test between the model including Bitou, *M. polana* abundance and habitat and the model including only Bitou and habitat showed that *M. polana* abundance made a significant contribution to the fit of both models). This shows that changes in the dipteran seed herbivore community are associated with the biocontrol agent's abundance and not with Bitou

abundance. In coastal forests and in the grouped habitat (dunes and headlands), average losses of 5.4 and 1.7 species of dipteran seed herbivores respectively occur at the highest levels of *M. polana* abundance sampled (Fig 4.2; see equation in Table 4.2).

Native parasitoid community

The best models of parasitoid abundance and species richness included effects of habitat and *M. polana* abundance (Table 4.2). As with herbivores, coastal forest consistently had significantly higher values of abundance and species richness than the three grouped habitats. *Mesoclanis polana* abundance had a significant negative effect on both parasitoid abundance and species richness (Fig 4.2). As in dipteran seed herbivores, a model including both Bitou and *M. polana* abundance showed no significant effect of Bitou abundance, and models without *M. polana* abundance had considerably higher BIC values. For both parasitoid abundance and species richness, the effect of the biocontrol agent was significant over and above the effect of Bitou (Table 4.2). In coastal forest and in the grouped habitats (dunes, headlands and heathlands), average losses of 5.9 and 0.7 species of parasitoids respectively occur at the highest levels of *M. polana* abundance sampled (see equation in Table 4.2).

Native plant community

No significant effects of the abundance of Bitou or *M. polana* were detected on species richness of native plants. These explanatory variables did, however, influence the abundance of native plants. The best model of abundance of native plants included abundance of Bitou and *M. polana*; the first had a significant negative effect and the second had a significant positive effect (Table 4.3). An F-test between the model including Bitou and *M. polana* abundance and a model including only Bitou abundance showed that *M. polana* abundance made a significant contribution to the fit of the model.

Discussion

Several studies have revealed how apparent competition via shared natural enemies can strongly affect population dynamics (Muller & Godfray 1997; Tompkins *et al.* 2000; Morris *et al.* 2001) and community structure (*e.g.* Morris *et al.* 2005). This study is the first in which the implications of apparent competition for the safety of the use of biocontrol agents are considered. Data here presented, clearly show that the biocontrol agent *M. polana* hosts a large number of abundant natural enemies, some of which are shared with native species, and that there is a significant negative relationship between *M. polana* abundance and local biodiversity of native herbivores and parasitoids. As this agent does not effectively reduce Bitou abundance (Noble & Weiss 1989), its introduction has led to more disadvantages than advantages for the native ecosystem.

Limitations

Although sampling took place in a limited period of 3 months, it coincided with one of the fruiting peaks of Bitou, and hence with a peak in the abundance of *M. polana.* Nevertheless, it is possible that *M. polana* has further impacts on species the activity period of which falls outside the sampling period. Moreover, given the methods used and the lack of previous records of native biodiversity, it would not be possible to detect if any species had already been driven to extinction. The results presented here relate only to biodiversity currently co-existing with the biocontrol agent, during the sampling period. In addition, identification of the exact link between parasitoid and host was not always possible. However, any limitations concerning host identification do not affect the assessment of the species richness or abundance of the different trophic levels, and so the interpretation of the models presented in this study is valid.

To quantify the impacts of a biocontrol agent fully, food webs should be constructed before release of the biocontrol agent, and then again after the agent is established. However, if the agent is already very widespread in the entire range of the target weed, as *M. polana* is, this is not possible. Moreover, this approach is only

possible if the agent becomes established and abundant, which does not always occur (Simberloff & Stiling 1996a), and if it does occur may take many years. Impact assessment studies over gradients of the weed and the biocontrol agent, such as this study, are the only alternative. Given the high correlation of the abundance of the agent with that of the weed, the statistical power available to detect a significant effect of the biocontrol agent over and above the effect of the weed is low, and hence only when the effect of the agent is strong (as it is here) can it be detected.

The food web

Several natural enemies were shared between *M. polana* and seed herbivores from native plants (Fig 4.1). The midge *Lestodiplosis* sp., a generalist predator (Gagné 1994) was the most abundant shared natural enemy. Dissections of the fruits would be needed to confirm that *Lestodiplosis* feeds on *M. polana*. However, given the high number of Bitou fruits reared (10,194 seed units), the high number of *Lestodiplosis* reared from them, the negative association between *Lestodiplosis* abundance and the lepidopteran seed herbivores reared from Bitou, and the positive association between *Lestodiplosis* and *M. polana* abundance (Table 4.1), it seems likely that *M. polana* is used as a food resource by *Lestodiplosis*.

As in Willis and Memmott (2005), no links with other herbivores were found for the most abundant parasitoid species of *M. polana* (Fig 4.1). It is possible that the original host species is not a seed herbivore, or has an activity period outside the sampling period. Alternatively, parasitoids may have used the newly acquired host, the biocontrol agent, to expand their range. However, it is also possible that the original host species was dropped from the parasitoids' diet or has been driven to local extinction due to elevated levels of attack by the highly abundant parasitoids. Since *M. polana* is now abundant throughout the range of Bitou, and no studies took place before the introduction of the biocontrol agent, these alternative hypotheses can no longer be tested.

Impact of M. polana *on native biodiversity*

The statistical models clearly demonstrate that, where Bitou makes up less than 50% of plant coverage, the most important factor affecting insect biodiversity is the abundance of Bitou's biocontrol agent and not that of Bitou itself (Table 4.2). Even in sub-optimal models of dipteran seed herbivore and parasitoid species richness and abundance, where Bitou was included, the effect of *M. polana* abundance was significant over and above the effect of Bitou abundance, but not *vice versa*. Thus, *M. polana* abundance is significantly negatively associated with the abundance and species richness of communities of insects reared from native plants (both dipteran seed herbivores and their parasitoids) (Fig 4.2). Direct competition between seed herbivores from native plants and *M. polana* is very unlikely to take place, as *M. polana* is highly specific to Bitou (Adair & Bruzzese 2000). As the food web approach revealed that natural enemies are shared between the biocontrol agent and other seed herbivore species, apparent competition is the most likely explanation for the observed negative statistical effect, especially given that dipteran seed herbivores were specifically affected. The midge *Lestodiplosis* is the most likely driver of apparent competition, as it is the most abundant natural enemy shared between *M. polana* and seed herbivores from native plants (Fig 4.1). However, a combination of shared natural enemies (*e.g.* parasitoids, predators and potentially pathogens) may be responsible for the decline of herbivores on native plants. The significant decrease in species richness and abundance of the parasitoids that exclusively attack seed herbivores of native plants is likely to be a result of the decline in the abundance of their hosts. Using the food web here presented, it would be possible to identify the native seed herbivores most likely to be affected by *M. polana,* and to test, under laboratory conditions, the impact of an increase of the abundance of the biocontrol agent on those native herbivore species, via shared natural enemies.

As Bitou is extremely invasive and highly competitive (Weiss & Noble 1984b), I expected it to have a negative influence on native plant abundance. However, the positive effect of *M. polana* on native plant abundance was surprising, as I expected the reduction in seed herbivores to have an effect only if plants were seed limited. As some of the most abundant *M. polana* parasitoids (*Eupelmus* (*Eupelmus*) sp., *Megastigmus* sp.

and *Torymoides kiesenwetteri* (Mayr)) are known to attack seed herbivores found on plants occupying different ecological niches in the study region (NHM 2007b; Ovruski *et al.* 2004), it is also possible that the positive effect detected here is due to further impacts on types of herbivores that are more likely to affect plant abundance. Further surveys on other ecological niches are needed to evaluate fully the potential for apparent competition between *M. polana* and native communities.

Implications for biological control

This study showed that the use of a highly specific biocontrol agent is significantly associated with the local loss of native species. Endemic species the range of which overlap completely with the range of the biocontrol agent (*i.e.* the eastern coast of Australia) are particularly vulnerable, as they are the species most likely to be driven extinct. A large number of Australian native insect species have not yet been described. However, 90% of plants and arthropods in Australia are likely to be endemic (Raven & Yeates 2007), so many endemic insect species may be endangered by this indirect effect. Since most biocontrol programmes involve several agents (Julien & Griffiths 1998), non-target impacts could accumulate, particularly if the agents are ineffective at reducing weed density but become abundant themselves. Furthermore, local losses can lead to regional losses, which may lead to extinctions (Finlay *et al.* 2006).

Invasive weeds such as Bitou may have much greater negative impacts on native communities (Weiss & Noble 1984b; French & Zubovic 1997; Lindsay & French 2006) than those demonstrated for *M. polana*. Therefore, provided a biocontrol agent is effective in suppressing the target weed, these positive effects may outweigh the type of negative impacts demonstrated here. Although, seed herbivores, such as *M. polana*, may be quite influential in plant dynamics, the strength of their impacts under various abiotic and biotic conditions is often unknown (Maron & Crone 2006).

In some situations, biological control may be the most cost-effective and least harmful method of pest management. However, the risk of negative non-target impacts must be considered. To avoid the introduction of ineffective biocontrol agents (with their increased risk of non-target effects), more investment is needed in pre-release studies in which the effectiveness of biocontrol agents in reducing weed abundance in

the invaded range is tested (Wirf 2006). Mathematical models are a powerful tool in this field (Barlow & Kean 2004; Buckley *et al.* 2005). If the potential for apparent competition is to be incorporated into these models, data must be collected on the natural enemies of the biocontrol agent in its native range, and on the natural enemies of related insects in the new range. The time a biocontrol agent takes to affect the abundance of the target weed should also be considered, as the longer a biocontrol agent is present and highly abundant, the higher the probability of non-target effects.

Concluding remarks

Although it has been argued that non-target effects of the use of highly specific biocontrol agents are occasional and predictable (Sheppard *et al.* 2006), this study reveals an example of a highly specific non-effective biocontrol agent, which, by remaining highly abundant in the ecosystem, led to the potential for apparent competition via shared natural enemies, and is also associated with highly significant impacts on native biodiversity. Therefore, more attention must be paid to the network of interactions which link biocontrol agents to native species, as any agent that is attacked by natural enemies can cause a cascade of changes through the food web, affecting species at several trophic levels. Community-scale analyses, such as that presented in this study, can be valuable tools in biocontrol research, as they provide the means to assess the negative impacts of introduced biocontrol agents fully, by clearly revealing how they integrate into the invaded community. Ideally, more collaboration is needed between weed biocontrol practitioners and network ecologists. Such collaborations could work to the advantage of both disciplines: if the most susceptible native species or guilds can be identified, the real costs and benefits of biological control can be taken into account when management decisions are made.

Tables and Figures

Table 4.1 Natural enemy (reared from Bitou) abundance models: comparison of the four best models obtained for each response variable (ordered by Bayesian Information Criterion, BIC; Akaike Information Criterion, AIC, values are also given and the best model according to each criterion is shown in bold). Explanatory variables used were: habitat, *M. polana* abundance, and abundance of Lepidoptera reared from Bitou. *P* values were obtained from a likelihood ratio test in which deviances with and without that term in the model were compared. ns – $P > 0.05$, blank cells represent terms not included in that model. *M. polana* range was 0-774 individuals per plot in grouped habitat and 330-2855 individuals per plot in coastal forest. **Hg** – grouped habitat, **Hcf** - coastal forest, **Mpol** – *M. polana* abundance. Correlation with *M. polana* abundance: *Lestodiplosis* sp. r=0.83; *Eupelmus* (*Eupelmus*) sp. r=0.71; *Megastigmus* sp. r=0.89 (grouped habitat), r=0.92 (coastal forest); *Pteromalus* sp. r=0.79. '–' variable not included in the model.

Response variable (Y)	Habitat	*M. polana* abundance	Lepidoptera abundance	BIC	AIC	residual df
Lestodiplosis sp. abundance log transformed, Gaussian error distribution	-	P = 0.0007	-	**36.9**	38.3	12
	ns	P = 0.0167	-	37.0	**37.7**	11
		P = 0.0020	ns	37.3	38.0	11
	ns	P = 0.0216	ns	37.9	38.0	10
Best model		**Ln (Y+1) = 0.8273 + 0.0011*Mpol**				
Eupelmus (*Eupelmus*) sp. abundance log transformed, Gaussian error distribution	-	P = 0.0004	-	**47.4**	**48.8**	12
	-	P = 0.0379	ns	49.3	50.0	11
	ns	P = 0.0057	-	49.8	50.5	11
	ns	ns	ns	51.3	51.4	10
Best model		**Ln (Y+1) = 0.5399 + 0.0018*Mpol**				
Megastigmus sp. abundance log transformed, Gaussian error distribution	ns	P = 0.0009	-	**46.9**	**47.6**	11
	-	P = 0.0003	-	47.0	48.4	12
	ns	P = 0.0236	-	47.9	48.6	11
	-	P = 0.0022	ns	48.2	48.9	11
Best model		**Ln (Y+1) = 0.3650Hg + 1.5683Hcf + 0.0014*Mpol**				
Pteromalus sp. abundance log transformed, Gaussian error distribution	-	P = 0.0006	-	**37.7**	39.1	12
	-	P = 0.0020	ns	38.1	**38.8**	11
	ns	P = 0.0082	-	40.2	40.9	11
	ns	P = 0.0111	ns	40.7	40.8	10
Best model		**Ln (Y+1) = 0.6005 + 0.0012*Mpol**				

Table 4.2. Insect abundance and species richness models: comparison of the four best models obtained for each response variable (ordered by Bayesian Information Criterion, BIC; Akaike Information Criterion, AIC, values are also given and the best model according to each criterion is shown in bold). *P* values were obtained from a likelihood ratio test in which deviances were compared with and without that term in the model. ns – $P > 0.05$, blank cells represent terms not included in that model. *M. polana* range was 0-774 individuals in grouped habitat and 330-2855 individuals in coastal forest. **Hg** – grouped habitat, **Hcf** - coastal forest, **Mpol** – *M. polana* abundance.

Response variable (Y)		Habitat	Bitou abundance	*M. polana* abundance	BIC	AIC	residual df
Seed herbivores of native plants	Abundance (log transformed) Gaussian error distribution	P = 0.0085	-	P = 0.0492	**65.6**	**65.9**	14
		ns	-	-	67.6	68.8	15
		P = 0.0134	ns	ns	68.4	67.9	13
		P = 0.0320	ns	-	68.5	68.8	14
	Best model	**Ln (Y+1) = 2.8409Hg + 5.7973Hcf -0.0012*Mpol**					
	Species Richness Quasi-Poisson error distribution	P = 0.0060	-	P = 0.0465	**119.6**	**120.0**	14
		P = 0.0158	ns	ns	128.5	128.0	13
		P = 0.0337	ns	-	132.4	132.7	14
		ns	-	-	133.1	134.2	15
	Best model	**Ln (Y) = 1.7594Hg + 2.9388Hcf -0.0006*Mpol**					
Dipteran seed herbivores from native plants	Abundance (log transformed) Gaussian error distribution	P = 0.0157	-	P = 0.0048	**41.9**	**42.8**	10
		P = 0.0223	ns	P = 0.0416	44.5	44.8	9
		ns	ns	-	48.2	49.1	10
		ns	-	-	50.2	51.6	11
	Best model	**Ln (Y+1) = 14.9034Hg + 4.0696Hcf -0.0014*Mpol**					
	Species Richness Poisson error distribution	P = 0.0092	-	P = 0.0043	**50.3**	**51.2**	10
		P = 0.0154	ns	P = 0.0259	52.7	53.0	9
		P = 0.0467	P = 0.0217	-	56.8	57.7	10
		ns	-	-	61.8	63.2	11
	Best model	**Ln (Y) = 1.056Hg + 2.1270Hcf -0.0012*Mpol**					
Parasitoids reared from native plants	Abundance (log transformed) Gaussian error distribution	P = 0.0015	-	P = 0.0216	**52.9**	**53.2**	14
		P = 0.0031	ns	P = 0.0485	55.4	54.9	13
		P = 0.0235	-	-	56.6	57.8	15
		P = 0.0161	ns	-	57.9	58.2	14
	Best model	**Ln (Y+1) = 0.7401Hg + 3.3560Hcf -0.0010*Mpol**					
	Species Richness (log transformed) Gaussian error distribution	P = 0.0005	-	P = 0.0079	**32.6**	**32.9**	14
		P = 0.0012	ns	P = 0.0234	35.1	34.6	13
		P = 0.0208	-	-	38.6	39.8	15
		P = 0.0105	ns	-	39.2	39.5	14
	Best model	**Ln (Y+1) = 0.5436Hg + 2.1856Hcf -0.0007*Mpol**					

Table 4.3 Native plant abundance models: comparison of the four best models (ordered by Bayesian Information Criterion, BIC; Akaike Information Criterion, AIC, values are also given and the best model according to each criterion is shown in bold). Bitou abundance range: 0-41%, *M. polana* abundance range: 0-2855 individuals per plot. *P* values were obtained from likelihood ratio F-tests comparing deviances with and without that term in the model. ns: $P>0.05$, blank cells represent terms not included in that model. **Mpol** – *M. polana* abundance.

Response variable (Y)	Habitat	Bitou abundance	*M. polana* abundance	BIC	AIC	residual df
Native plant abundance arcsin transformed, Gaussian error distribution	-	P = 0.0046	P = 0.0208	**11.0**	**11.3**	14
	ns	P = 0.0047	P = 0.0475	13.1	12.6	13
	ns	P = 0.0359	-	15.6	15.9	14
	-	ns	-	14.8	16.0	15
Best model	**arcsin Y = 1.0133 - 0.0362*Bitou + 0.0004*Mpol**					

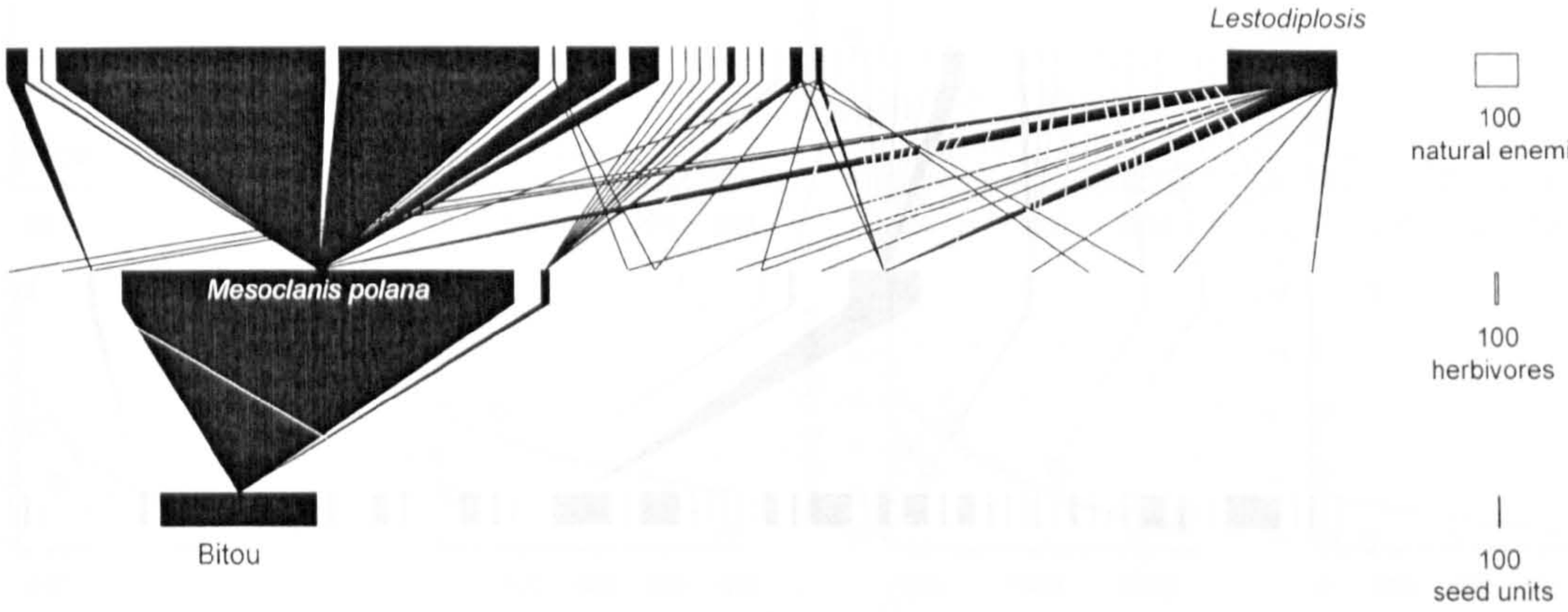

Figure 4.1 Plant-seed herbivore-natural enemy web for NSW, Australia (all data combined). Plant and natural enemy species are represented by rectangles. If their interactions between seed herbivores and natural enemies from a given plant species could not be clearly identified, all seed herbivores from the plant species from which the natural enemy was reared were grouped together, and each group is represented by a rectangle. The widths of the rectangles are proportional to the species' abundance at the field site, and the size of the triangles connecting the rectangles represents the frequency of interactions at the field site. All interactions with Bitou are shown in black. Lists of seed herbivore, plant and natural enemy species per group are provided in Tables S4.4-6.

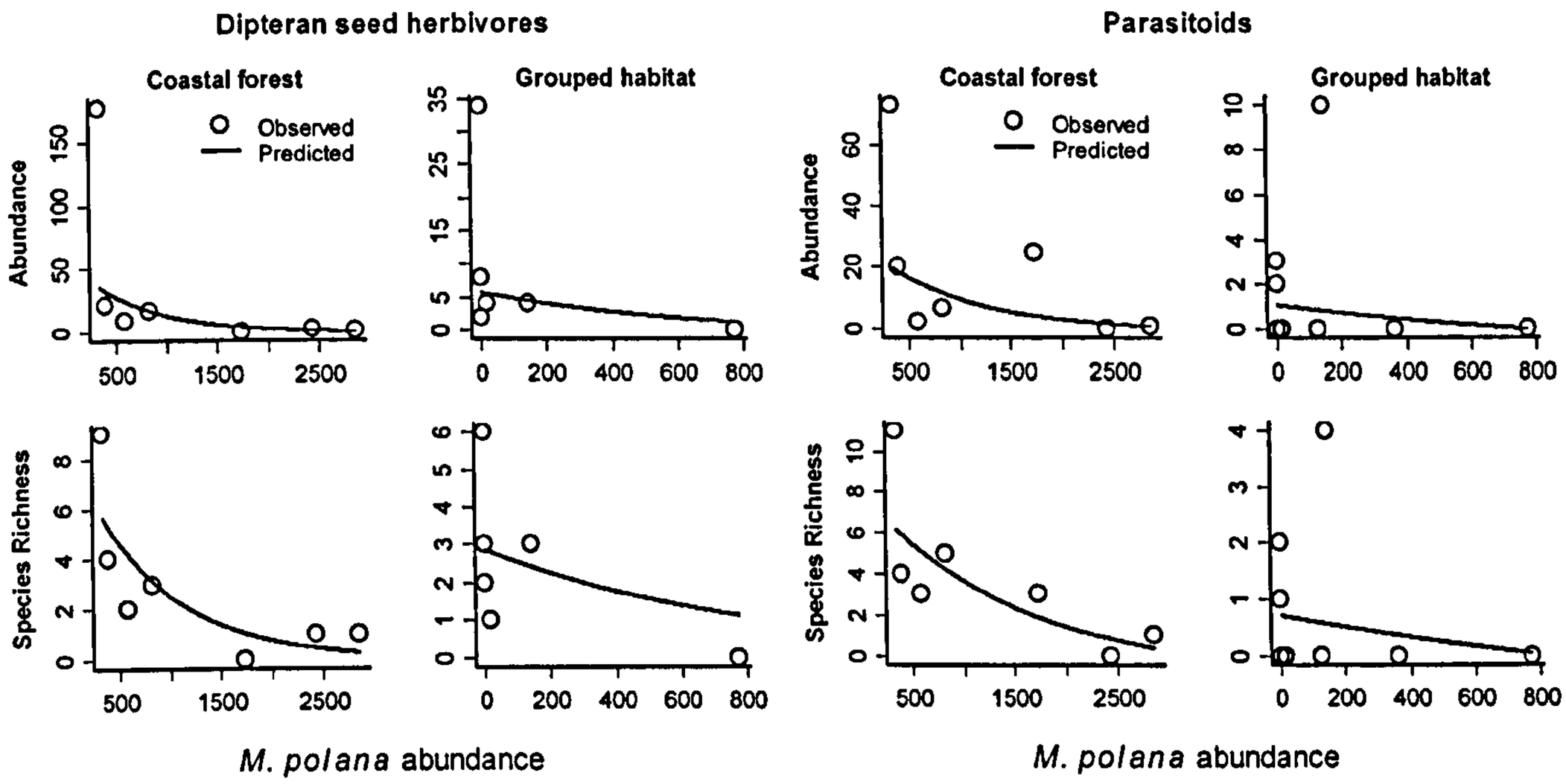

Figure 4.2 Models of abundance and species richness of dipteran seed herbivores and parasitoids reared from native plants. Graphs show the observed data with model predictions of the significant negative effect of *M. polana* abundance for each habitat. Model equations and model selection steps are provided in Table 4.2. Dipteran seed herbivores: abundance model R^2=0.58, species richness model R^2=0.50. Parasitoids: abundance model R^2=0.53, species richness model R^2=0.59.

Supplementary material

Table S4.1 Grid references (UTM) of the 17 study plots and of the two plots where additional sampling was carried out.

Habitat	Plot coordinates	Bitou abundance (% coverage area)
Dunes	68219N5598E	0.2
Coastal forest	68203N5592E	40.8
Headland	68110N5591E	2.0
Coastal forest	67899N5452E	19.1
Coastal forest	67876N5439E	18.9
Heathland	67771N5430E	0.0
Headland	67765N5444E	3.1
Coastal forest	67760N5431E	20.2
Dunes	67752N5433E	29.2
Coastal forest	67702N5394E	8.3
Dunes	67683N5382E	3.2
Dunes	67545N5332E	0.0
Coastal forest	67522N5340E	19.8
Coastal forest	67501N5358E	11.6
Heathland	67395N5347E	6.1
Heathland	67386N5342E	0.4
Heathland	67380N5346E	3.1
Additional sampling		
Headland	6738N335E	-
Interior Forest	6864N497E	-

Table S4.2 Plant species of the food web. The order of the species corresponds to the order in the food web diagram (Fig 2.1). * - introduced species.

Plant species	Plant order	Plant family	Number of seed units
Carpobrotus glaucescens (Haw.) Schwantes	1	Aizoaceae	66
Tetragonia tetragonioides (Pall.) Kuntze	2	Aizoaceae	400
Platysace ericoides (Sieber ex Spreng.) Norman	3	Apiaceae	55
Asteraceae sp1	4	Asteraceae	12
Asteraceae sp2	5	Asteraceae	12
Asteraceae sp3	6	Asteraceae	8
Asteraceae sp4	7	Asteraceae	23
Asteraceae sp5	8	Asteraceae	12
Asteraceae sp6	9	Asteraceae	80
Bidens pilosa L. *	10	Asteraceae	532
Bitou - *Chrysanthemoides monilifera* ssp. *rotundata* (L.) T. Nord *	11	Asteraceae	10194
Conyza bonariensis (L.) Cronquist *	12	Asteraceae	185
Melanthera biflora (L.) Wild	13	Asteraceae	3
Senecio madagascariensis Poir. *	15	Asteraceae	38
Senecio pinnatifolius A.Rich.	16	Asteraceae	1058
Sonchus oleraceus L.	17	Asteraceae	215
Xerochrysum bracteatum (Vent.) Tzvelev	18	Asteraceae	27
Casuarina equisetifolia L.	19	Casuarinaceae	25
Gloriosa superba L. *	20	Liliaceae	48
Ipomoea pes-caprae brasiliensis (L.) Sweet	21	Convolvulaceae	1248
Diplocyclos palmatus (L.) Jeffrey	22	Cucurbitaceae	391
Hibbertia sp1	23	Dilleniaceae	57
Hibbertia sp2	24	Dilleniaceae	34
Hibbertia sp3	25	Dilleniaceae	49
Hibbertia scandens (Willd.) Gilg	26	Dilleniaceae	3798
Epacris microphylla R.Br.	28	Ericaceae	78
Leucopogon parviflorus (Andrews) Lindl.	29	Ericaceae	2707
Bossiaea heterophylla Vent.	30	Fabaceae	180
Fabaceae sp1	31	Fabaceae	33
Fabaceae sp2	32	Fabaceae	31
Macroptilium atropurpureum (DC.) Urb.	33	Fabaceae	90
Sophora tomentosa L.	34	Fabaceae	29
Stephania japonica (Thunb.) Miers	35	Menispermaceae	834
Austromyrtus dulcis (C.T.White) L.S.Sm.	36	Myrtaceae	410
Leptospermum laevigatum (Gaertn.) F.Muell.	37	Myrtaceae	2646
Leptospermum sp.	38	Myrtaceae	34
Melaleuca nodosa (Sol. ex Gaertn.) Sm.	39	Myrtaceae	810
Melaleuca quiquenervia (Cav.) S.T.Blake	40	Myrtaceae	1624
Dianella congesta R.Br.	41	Phormiaceae	257
Rivina humilis L. *	42	Phytolaccaceae	1070
Poaceae sp1	43	Poaceae	279
Poaceae sp2	44	Poaceae	156
Spinifex sericeus R.Br.	45	Poaceae	19
Banksia integrifolia	46	Proteaceae	291
Banksia oblongifolia Cav.	47	Proteaceae	116
Banksia serrata L.f.	48	Proteaceae	527
Hakea actites W.R.Barker	49	Proteaceae	337
Persoonia sp1	50	Proteaceae	108
Persoonia adenantha Domin	51	Proteaceae	165
Persoonia virgata R.Br.	52	Proteaceae	1639
Cupaniopsis anacardioides (A.Rich.) Radlk.	53	Sapindaceae	388
Smilax australis R.Br.	54	Smilacaceae	3

Lycopersicon esculentum Mill.*	55	Solanaceae	7
Solanum americanum Mill.	56	Solanaceae	3638
Solanum seaforthianum Andrews *	57	Solanaceae	117
Pimelea linifolia Sm.	58	Thymelaeaceae	142
Lantana camara L. *	59	Verbenaceae	48
Ricinocarpos pinifolius Desf.	60	Euphorbiaceae	94

Table S4.3 Seed herbivore species of the food web. The order of the species corresponds to the order in the food web diagram (Fig 2.1). * - seed herbivore groups which share natural enemies with *M. polana*. ** - dipteran seed herbivores, reared from native plants, that only occur when *M. polana* is present at low abundance. Col – Coleoptera; Dip – Diptera; Hym – Hymenoptera; Lep – Lepidoptera.

Host Plant	Seed herbivore group	Seed herbivore species	Order	Number of specimens
Carpobrotus glaucescens	1*	Unidentified seed herbivore	Not identified	0.1
Asteraceae sp3	2	Lep sp1	Lep	4
Asteraceae sp6	3*	Unidentified seed herbivore	Not identified	1
Bidens pilosa	4*	*Dyoxina sorocula* (Wiedemann)	Dip: Tephritidae	193
Xerochrysum bracteatum	4*	*Dyoxina sorocula* (Wiedemann)	Dip: Tephritidae	0.1
Bitou – *C. m.* ssp. *rotundata*	5	*Mesoclanis polana* (Munro)	Dip: Tephritidae	10576
	6	Lep sp2	Lep	78
		Lep sp3	Lep	74
		Lep sp4	Lep	23
		Lep sp5	Lep	21
		Lep sp6	Lep	4
		Lep sp7	Lep	8
		Lep sp8	Lep	7
		Lep sp9	Lep	1
Conyza bonariensis	7	Lep sp10	Lep	1
Senecio madagascariensis	8	Lep sp11	Lep	1
Senecio pinnatifolius	9*	*Trupanea prolata* Hardy and Drew	Dip: Tephritidae	10
		Lep sp12	Lep	1
Sonchus oleraceus	10*	Unidentified seed herbivore	Not identified	0.1
Xerochrysum bracteatum	11*	Lep sp13	Lep	0.1
		Curculionidae sp1	Col: Curculionidae	0.1
Casuarina equesetifolia	12	Lasiopteridi sp.	Dip: Cecidomyiidae	3
Gloriosa superba	13*	Unidentified seed herbivore	Not identified	0.1
Ipomoea pes-caprae brasiliensis	14*	*Atherigona* sp. **	Dip: Muscidae	3
		Euphranta lemniscata Enderlein **	Dip: Tephritidae	1
		Helcomyzidae sp.	Dip: Helcomyzidae	4
		Gaurax sp. **	Dip: Chloropidae	1
Diplocyclos palmatus	15	*Atherigona* sp **	Dip: Muscidae	11
		Bactrocera bryoniae (Tryon) **	Dip: Tephritidae	24
		Bactrocera chorista (May) **	Dip: Tephritidae	89
		Gaurax sp. **	Dip: Chloropidae	1
		Lep sp14	Lep spS2	1
Hibbertia sp2	16*	Unidentified seed herbivore	Not identified	0.1
Hibbertia scandens	17*	Heleomyzidae sp.	Dip: Heleomyzidae	3
		Lonchaeidae sp. **	Dip: Lonchaeidae	5
		Gaurax sp.1 near *luteicornis* Malloch **	Dip: Chloropidae	11
		Gaurax sp. 2 near *luteicornis* Malloch **	Dip: Chloropidae	52
		Gaurax sp. near *quadristriata* Becker **	Dip: Chloropidae	48
		Lep sp15	Lep	6
		Col sp1	Col	24
		Col sp2	Col	3
		Col sp3	Col	4
		Col sp4	Col	18
		Col sp5	Col	3
		Eurytoma sp1	Hym: Eurytomidae	577
		Austrophotismus daicles (Walker)	Hym: Eurytomidae	1175
Leucopogon parviflorus	18	*Asphondylia* sp.	Dip: Cecidomyiidae	0.1
Fabaceae sp2	19	*Eurytoma* sp2	Hym: Eurytomidae	0.1
	20		Col	
Melaleuca nodosa		Coleoptera sp6		1

Melaleuca quiquinervia	21*	Lep sp16	Lep	7
		Chrysomelidae sp.	Col: Chrysomelidae	154
		Col sp7	Col	1
		Curculionidae sp2	Col: Curculionidae	9
		Eurytoma sp3	Hym: Eurytomidae	1
Dianella congesta	22	Unidentified seed herbivore	Not identified	0.1
Rivina humilis	23*	Ephydridae sp **	Dip: Ephydridae	1
		Unidentified seed herbivore	Not identified	0.1
Spinifex sericeus	24	Col sp8	Col	1
Banksia integrifolia	25	Lep sp17	Lep	13
		Lep sp18	Lep	2
		Lep sp19	Lep	3
		Col sp9	Col	30
		Curculionidae sp3	Col: Curculionidae	12
		Curculionidae sp4	Col Curculionidae	10
		Tenebrionidae sp1	Col: Tenebrionidae	3
		Hym sp1	Hym	1
Banksia oblongifolia	26*	Hym sp2	Hym	1
Banksia serrata	27	Curculionidae sp5	Col: Curculionidae	8
		Curculionidae sp6	Col: Curculionidae	5
		Curculionidae sp7	Col: Curculionidae	10
		Curculionidae sp8	Col: Curculionidae	11
		Curculionidae sp9	Col: Curculionidae	5
		Curculionidae sp10	Col: Curculionidae	18
		Curculionidae sp11	Col: Curculionidae	16
		Tenebrionidae sp2	Col: Tenebrionidae	6
		Hym sp3	Hym	2
		Hym sp4	Hym	2
Hakea actites	28	Hym sp5	Hym	1
Cupaniopsis anacardioides	29	Lep sp20	Lep	0.1
		Unidentified seed herbivore	Not identified	0.1
Solanum americanum	30	Col sp10	Col	1
		Unidentified seed herbivore	Not identified	0.1
Pimelea linifolia	31*	Dip sp1	Dip	4
Lantana camara	32	Lep sp21	Lep	3
		Lep sp22	Lep	1
		Lep sp23	Lep	1
		Lep sp24	Lep	2

Table S4.4 Parasitoid species of the food web. Seed herbivore groups are defined in Table S4.3. The order of the species corresponds to the order in the food web diagram (Fig 2.1). * - parasitoid species, reared from native plants, that only occur when *M. polana* is present at low abundance. Dip – Diptera; Hym – Hymenoptera

Parasitoid order	Parasitoid species	Order: Family	Host seed herbivore group	Number of interactions
1	*Aprostocetus* sp1	Hym: Eulophidae	4	37
			5	11
2	*Aprostocetus* sp2	Hym: Eulophidae	5	3
3	*Eupelmus* (*Eupelmus*) sp.	Hym: Eupelmidae	5	592
4	*Megastigmus* sp1	Hym: Torymidae	5	441
5	Opiinae sp1	Hym: Braconidae	5	2
			9	1
			10	1
6	*Pteromalus* sp.	Hym: Pteromalidae	5	108
7	*Torymoides kiesenwetteri* (Mayr)	Hym: Torymidae	5	64
8	Aphelinidae sp.	Hym: Aphelinidae	6	2
9	Cheloninae sp.	Hym: Braconidae	6	1
10	*Eurytoma* sp3	Hym: Euytomidae	6	1
11	Phygadeuontinae sp.	Hym: Ichneumonidae	6	1
12	Rogadinae sp.	Hym: Braconidae	6	17
13	Scelionidae sp.	Hym: Scelionidae	6	1
14	*Tossinola* sp.	Hym: Ichneumonidae	6	3
15	*Systasis* sp.	Hym: Pteromalidae	8	2
16	Dolichogenidae sp1	Hym: Dolichogenidae	6	22
			10	2
			17	2
			25	1
17	*Leptopilina* sp.	Hym: Eucoilidae	5	2
			14	1
			17	9
			23	1
18	Braconinae sp1 *	Hym: Braconidae	15	7
19	Chalcidoidea sp1 *	Hym	17	2
20	Chalcidoidea sp2 *	Hym	17	4
21	Eurytomidae sp. *	Hym: Eurytomidae	17	1
22	Eurytoma sp4 *	Hym: Eurytomidae	17	8
23	Hym sp1 *	Hym	17	2
24	Hym sp2 *	Hym	17	4
25	*Megastigmus* sp2 *	Hym: Torymidae	17	48
26	*Megastigmus* sp3 *	Hym: Torymidae	17	1
27	Platygasteridae sp. *	Hym: Platygasteridae	17	2
28	Tetrastichinae sp2 *	Hym: Tetrastichidae	18	1
29	*Bootanomyia* sp.	Hym: Torymidae	21	19
30	Chalcidoidea sp3	Hym	21	5
31	*Eurytoma* sp2	Hym: Eurytomidae	21	4
32	Braconidae sp1 *	Hym: Braconidae	22	1
33	*Megastigmus* sp4	Hym: Torymidae	23	1
34	Braconinea sp2 *	Hym: Braconidae	25	1
35	Chalcidoidea sp3 *	Hym	25	1
36	Ichneumoidea sp. *	Hym	25	3
37	*Tineobius* sp. *	Hym: Eupelmidae	25	2
38	*Euderus* sp. *	Hym: Eulophidae	27	2
39	*Elasmus* sp. *	Hym: Elasmidae	29	1
40	Tetrastichinae sp3 *	Hym: Eulophidae	30	1
41	Chalcidoidea sp4 *	Hym	31	2
42	Agathidinae sp.	Hym	32	1

43	*Lestodiplosis* sp.	Dip: Cecidomyiidae	1	0.1
			3	1
			4	2
			5	136
			9	5
			13	3
			14	1
			16	1
			17	81
			21	2
			26	1
			31	8

Chapter 5

DISCUSSION

Chapter 5

Discussion

Overview: Invasive plants can have negative consequences on both conservation and agro-economy, and are the target of management strategies worldwide. Despite this, studies evaluating the community level effects of the integration of exotic plant species into native systems are rare, as are studies on how food web topology influences the way these effects are propagated through food webs.

In the three data chapters of this thesis I used a food web approach to evaluate how exotic species are integrated in native communities. The first two chapters focused on exotic plant species, and the last on an exotic seed herbivore. By providing quantitative information on trophic relationships, the studies presented in this thesis give insights on the propagation of alien species impacts through food webs. Chapters 2 and 3 show that impacts of exotic plant species on the native plant communities are not always representative of the impacts on higher trophic levels. Moreover, using a food web approach, Chapters 2 and 4 highlight the potential negative effects of management measures on the native systems, such as reduced pollination services, or a disruption of regulatory top-down mechanisms by natural enemies.

I will now synthesize the major findings of this thesis in relation to impacts of invasive species and consequences of management measures, discussing the utility of food webs for assessing and predicting such impacts.

Impacts of invasive plants at a community level

Most studies that evaluate impacts of invasive plants focus exclusively on the plant level (*e.g.* Hulme & Bremner 2006; Flory *et al.* 2007) which is relatively straightforward to assess. However, the outcomes for higher trophic levels can be different from those detected at plant level, with impacts being accentuated or even opposite. For example, in Chapter 3, where the invasive plant *G. shallon* in UK heathlands was used as a case study, a food web approach, involving three trophic levels, revealed negative effects in all levels analysed. However, impacts detected at plant level were not representative of the outcomes detected on insect communities, with some herbivores (specialists) being significantly more affected than their resources, while other herbivores (generalists) are not affected at all. Moreover, when generalist insect species are able to use exotic species (plants or insects) as a resource, they may benefit from an invasion. Such an effect was detected in Chapter 4 where natural enemies that used the introduced biocontrol agent, *M. polana*, as resource increased in abundance while parasitoid species which were not able to use *M. polana* as resource were negatively affected by an increase in abundance of the biocontrol agent. Exotic species can become an important part of generalist species diet, as shown in Chapter 2 (where plant-pollinator food webs involving rare native plants and exotic plants are analysed) for ants. My results support those of a previous study of a generalist pollinator community which benefited from the presence of a highly invasive weed, *Impatiens gladulifera* (Lopezaraiza-Mikel *et al.* 2007). In such cases, effects at plant level can be contrary to the effects on consumer communities. Therefore, in order to understand the full impact of invasive species it is important to assess their impact at a whole-community level, involving several trophic levels.

There are some examples of studies that evaluate impacts of invasive plants on communities of higher trophic levels such as soil invertebrates or birds (*e.g.* French & Zubovick 1997; Lindsay & French 2006; Gratton & Denno 2005). However these studies do not provide information on how species are trophically linked within a community. As habitat disturbances may lead to important changes in food web structure (Tylianakis *et al.* 2007) evaluating trophic relations between species is essential for understanding the impacts of invasive species. For example, Chapter 3 revealed that the presence of an invasive species lead to a more generalized and even

(*i.e.* species in the ecosystem have more similar abundances to each other and consumers feed equally on the available resources) food web structure, and to a loss of trophic diversity at herbivore and parasitoid level. As exotic species can be an important resource for generalist pollinators (Chapter 2), such changes may also occur in pollination systems. In fact, casual observations of flower visitors to plants in the heathland study plots (Chapter 3), showed that *G. shallon* flowers are frequently visited by two dominant pollinators, *Apis melifera* and *Bombus terrestris/lucorum*. Native plant species were visited by a more diversified set of flower visitors. *A. melifera* and *B. terrestris/lucorum* appeared to benefit from increased on weed abundance, enhancing their proportion in the overall flower visitor community. However, further sampling is required to test the statistical significance of this result.

My research has demonstrated that species with different trophic requirements are differentially affected by invasive species. Thus, future work on the impact of invasive plants should include information on trophic interactions focusing not only on loss of species diversity, but also on loss of trophic diversity.

Consequences of management measures

Exotic plant species can have a profound impact on natural habitats and it is important to find ways to quickly and effectively manage them in a variety of ecosystems. However, it is also important to consider possible negative impacts of management measures on native ecosystems, such as non-target effects that arise as a consequence of exotic species removal (Chapter 2), or from the management method itself (Chapter 4).

Non-target effects of exotic species removal

Invasive plant species may have been included in a native system for a long enough period of time to out-compete native species and become dominant. Consequently, they have become integrated into the native food web, which changes and adapts itself to the presence of the new exotic species. Integration into a native network of interactions can

occur *via* generalist species of consumers, such as pollinators (Chapter 2), or parasitoids (Chapter 4). Changes to the native system may include generalization and homogenization of the structure of food webs (see Chapter 3 as an example).

When invasive plant populations stabilize (*i.e.* not expanding into further areas) in the native system, or if they are a consequence of, rather than a driver of biodiversity change (Gurevitch & Padilla 2004; MacDougall & Turkington 2005; Thomas & Reid 2007), maximum damage to that native system has probably been achieved. Therefore, removing an often large, functional component of the system (albeit an invasive species), may bring further negative effects to native biodiversity (Zavaleta *et al.* 2001). For example, an invasive plant may provide important habitat and hence have a beneficial effect towards native species that co-exist in the invaded habitat: Buckley *et al.* (2006) highlighted the fact that several exotics are used as a food resource by native birds, and there may be a conflict between the control of invasive species and bird conservation and Moragues & Traveset (2005) along with Lopezaraiza-Mikel *et al.* (2007) revealed that, by providing important resources for pollinator communities, exotic species lead to facilitation of pollination of native plat species. Chapter 2 adds a further example to this list, highlighting the importance of alien plants to the maintenance of populations of generalist pollinators that are essential for the pollination of rare plant species. In such cases, rapid removal of the invasive species may act as an added disturbance.

Non-target effects of management methods

Generalist natural enemies are common in all communities, and hence indirect non-target effects (such as those revealed in Chapter 4), may occur with other established biocontrol agents. Exotic biocontrol agents (as with exotic plants), that become established and abundant in native system are integrated in native food webs and can lead to changes in food web structure. In the case of *M. polana* (Chapter 4), increases in abundance of this agent lead to an increase in the abundance of generalist natural enemies that were able to include this biocontrol agent in their diet. As these parasitoid species were predominately reared from *M. polana*, it is likely that they are highly dependent on this biocontrol agent rather than alternate hosts. In this case, rapid removals of the target weed, and consequently of the biocontrol agent, could lead to a

negative impact on these native parasitoids. If, however, native vegetation is successfully re-established, and the native seed-herbivores are able to recover their abundance and diversity, more specialized natural enemies may also recover with a successful restoration being achieved. Therefore, it is important to understand the community wide effect of invasive plants and of their management plans, to ensure that the conservation or biodiversity goals are met.

Food webs as a tool for assessing and predicting impacts

By providing quantitative information on trophic relations, food webs, such as those presented in this thesis, can be used to generate testable hypotheses based on the patterns of interactions (Memmott 1999). By analysing food webs involving the biocontrol agent, *M. polana*, Willis and Memmott (2005) predicted that apparent competition between the agent and native seed-herbivore communities was likely to occur. Based on this information, the study presented in Chapter 4 was developed, and negative effects on native dipteran seed-herbivores were detected, supporting Willis and Memmott's predictions, albeit via a different species (the predator *Lestodiplosis* instead of parasitoid species).

Other studies (*e.g.* Dunne *et al.* 2002, Memmott *et al.* 2004) used qualitative information on diet breath to use food webs as a predictive tool by simulating the consequences of species extinctions. Similar simulations can be used to predict the consequences of species loss due to exotic plant invasion, as well as to predict consequences of the removal of exotic species. However, to generate more accurate predictions, such studies should consider the importance of each resource species to a consumer diet (*i.e.* quantitative food webs), so general rules on adaptation ability and extinction thresholds of species can be included in simulations (see Chapter 2 as an example). As discussed in Chapter 3, in the case of *G. shallon* impacts on plant-herbivore-parasitoid community, adaptation ability is related to diet breath and can have a strong influence on the propagation of impacts. However, it is still not clear how frequently diet breath is the most important regulating factor of impact propagation (Fox 2007). Meta-analyses have compared several studies on trophic cascades (Shurin *et al.* 2002; Borer *et al.* 2005) with little success in defining general rules on the

propagation of impacts that could be broadly applicable to other systems (Borer *et al.* 2005). Studies included in such meta-analyses are, however, extremely variable in terms of ecosystems analyzed (*e.g.* aquatic *vs.* terrestrial ecosystems) and sampling methodology. Moreover, they mostly focus on trophic chains, and do not consider the complexity of interactions that characterizes the vast majority of natural systems.

Concluding remarks

Introduction of exotic species in new environments will continue and may even increase in frequency in the future. New pest species outbreaks are likely to occur, leading to further negative impacts and hence presenting more challenges for invasive species management. The work described in this thesis has shown that food webs may allow ecologists to better evaluate the impacts of invasive species and their management. By assessing impacts across several trophic levels, I give new insights on food web dynamics and provide ideas for the use of food webs as a predictive tool. More specifically the main conclusions of this work were:

1) Exotic plants made up an important part of the diet of certain generalist flower visitors (ants), which are essential for the pollination of rare inconspicuous plants, such as *T. glauca*. Therefore, management measures involving removal of alien plants should consider possible negative impacts on rare plants through changes in insect populations.

2) Consumer diet breadth affected propagation of impacts of the invasive *G. shallon*, leading to changes in the abundance and species richness of insect communities but also in the food web structure. Therefore, to fully evaluate impacts of invasive species it is important to consider all trophic levels and to consider trophic diversity within each level.

3) Highly specific biocontrol agents can share natural enemies with native seed herbivore species. Moreover, I present evidence of decline in both species richness

and abundance native insect communities along a gradient of biocontrol agent abundance. The most likely cause for these declines is apparent competition.

Where should we go from here?

This thesis raises several questions and highlights three main areas in need of further research:

1) How does the loss of trophic diversity and changes in food web evenness affect the functioning of ecological communities? Community level consequences of plant invasions, such as the loss of trophic diversity, also profoundly alter the evenness of species abundance and species interactions in ecosystems. Although an increase in interaction evenness has been associated with higher "quality" of ecosystems (Tylianakis *et al.* 2007), in the cases presented here it comes as a result of disturbance. Further studies are needed to understand the causes and long-term consequences of changes in evenness of food webs.

2) How widespread are indirect non-target effects of introduced biocontrol agents? Throughout this thesis the diversity of outcomes that result from ecosystem management measures has been highlighted. To make sure that the conservation goals are met, future management plans should consider the full network of trophic interactions so that all possible short-term and long-term consequences are considered. The inclusion of survey methods that use a food web approach into future programmes that monitor success and establishment of biocontrol agents could also evaluate how frequently biocontrol agents are likely to lead to indirect negative effects on native biodiversity. Such measures would require greater collaboration between weed biocontrol practitioners and network ecologists.

3) How can a food web approach be utilized as a predictive tool for conservation biology? Predicting how ecosystems will change as a result of disturbances is a major challenge in ecology. The definition of general rules that can be incorporated in predictive studies such as Dunne *et al.* (2002) or Memmott *et al.*

(2004) would provide the basis for the use of food webs as predictive tools in conservation biology. If accomplished, this would provide a valuable tool for predicting the consequences of not only of species invasion, but also of management measures. Therefore, more studies that test the influence of diet breath on regulation of bottom-up trophic cascades in further ecosystems are needed, as well as on the effects of apparent competition on top-down trophic cascades. These *in silico* predictions must then be tested in the field by manipulating real-life food webs, or looking at variations in food webs along gradients of disturbance.

Understanding how natural systems work is an immense challenge that involves many small steps. By applying a food web approach to examine the impacts and management of invasive species the work here presented provides some insights relevant to the field of conservation ecology.

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